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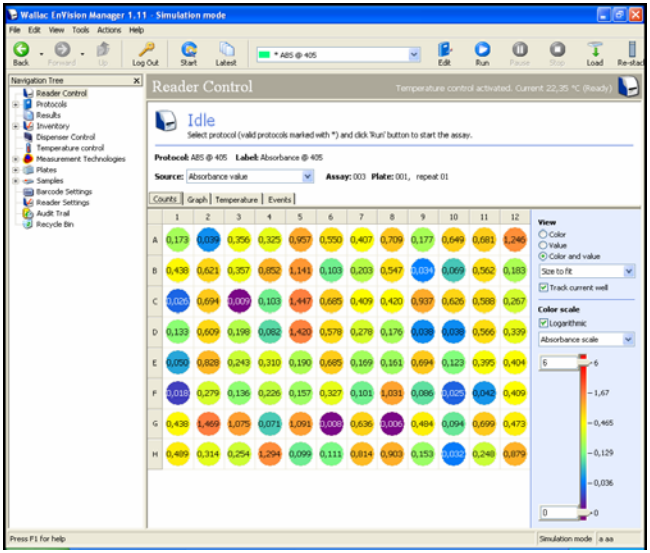
User manual

EnVision™

Software version 1.12


Wallac EnVision Manager 1.11 - Simulation mode
File Edit View Tools Actions Help
Reader Control
Idle
Select protocol (valid protocols marked with *) and click 'Run' button to start the assay.
Protocol: ABS @ 405 Label: Absorbance @ 405
Source: Absorbance value Assay: 003 Plate: 001, repeat 01
Counts | Graph | Temperature | Events
View
Color
Value
Color and value
Track current well
Color scale
Logarithmic
Absorbance scale
Press F1 for help
Simulation mode

PerkinElmer Life and Analytical Sciences, Wallac Oy, P.O. Box 10, FIN-20101 Turku, Finland.
Tel: 358-2-2678111. Fax: 358-2-2678357 Website: www.perkinelmer.com

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Chapter 1
Introduction

Introduction

The EnVision™ high throughput screening microplate reader from PerkinElmer Life and Analytical Sciences is a complete platform for quantitative detection of light-emitting or light absorbing markers. It is suitable for measurement of fast or glow luminescence, absorbance, fluorescence intensity, fluorescence polarization ([FP]^{2}™), high-sensitivity time-resolved fluorometry (DELFIA®) and homogeneous time-resolved fluorometry (LANCE™).

Very high sensitivity measurements can be made with the Ultra Sensitive luminescence option.

A laser-equipped model allows AlphaScreen measurements to be made. HTS AlphaScreen allows faster throughput than standard AlphaScreen.

Another option with an external laser enables enhanced time-resolved fluorescence measurements.

The monochromator option allows a wide range of wavelengths for absorption measurements. The dual monochromator option allows also wavelength selection for both excitation and emission light in fluorescence intensity measurements.

EnVision is a very compact, small footprint bench top unit with features such as shaking, reading from above or below and scanning.

Also available are a dispenser with up to two pumps and temperature control of the instrument chamber.

The software is a 32-bit application running under Windows XP® or Windows Vista®. Output can be to a file on the PC and/or to a laser printer or on the network.

Figure 1. Wallac EnVision with a stacker

Figure 2. Wallac EnVision without a stacker

Software installation

Note! Installation must be done only by PerkinElmer service personnel.

Note! The following instructions assume that EnVision, the CAN-card or optional control unit, PC and printer have been connected up and switched on (see the installation instructions in the Instrument manual).

1. Load the software CD.
2. The setup loader will start automatically. This will guide you through the installation.
3. When you have completed the installation you should remove the CD from the PC.

Start up

1. Switch on EnVision.
2. If you have a control unit, switch it on. This is a separate CPU used to run the instrument software. In an alternative configuration the job is handled by the workstation PC without a separate control unit.
3. Wait until the red light on the EnVision panel is steady (not blinking). This takes a couple of minutes.
4. Switch on the user PC.
5. Start Wallac EnVision Manager by clicking the EnVision icon.

Caution! For users with the Stacker option - keep your hands away from the stacker area when the software is started or restarted. The rods in the stacker will come up during the initialization process.

Shut down

1. To shut the loading door, press the LOAD button.
2. Close Wallac EnVision Manager by clicking **File** and **Exit**, or closing the window in the usual way. You will be given a choice to shut down either the workstation software only or the whole EnVision software. Choose the option you want and click **OK**.
3. Shut down the user PC.
4. If you have a control unit, press the ON/OFF button to shut it down. Wait while the light is flashing on the control unit (two or three minutes). Only if this button has not ceased flashing after several minutes should you press the same button again.
5. Shut down EnVision.

Note! If you are not going to use EnVision for a while, you can leave the power to the various units switched on but you should shut the loading door.

Using this manual

This manual describes the main steps to be followed when running samples on EnVision. It assumes that protocols have been set up and that all the needed components (filters etc.) have been installed.

Please refer to the Reference manual for detailed information about all types of parameter setting.

Part 1 Introduction
Tells you how the system works in general.

Part 2 Operation
Takes you through use of the **Assay Start Wizard** including optimization and proceeding to running assays.

Part 3 Results
Describes result viewing and output.

Part 4 Advanced Optimization
Explains some of the additional steps involved in optimization if you choose to use the **Advanced** selection in the **Assay Start Wizard**.

Part 5 Dispenser Control
Describes the software parameters used to control operation of the dispenser option. Gives guidelines for daily use of the dispenser option. Please refer to the Instrument manual for routine maintenance of the dispenser. ***Tip Mount*** and **Temperature Control** settings are described in the Reference manual.

Conventions used

Buttons or other software items to be clicked with the mouse are in bold text e.g. **File**.

Items in the main Navigation Tree are in bold italic e.g. ***Protocols***.

Buttons on the instrument that need to be pressed are in block letters e.g. START.

Note! The term "AlphaScreen" refers to both standard AlphaScreen and HTS AlphaScreen unless a distinction is specifically made. The term "special luminescence" refers to Enhanced luminescence and Ultra Sensitive luminescence.

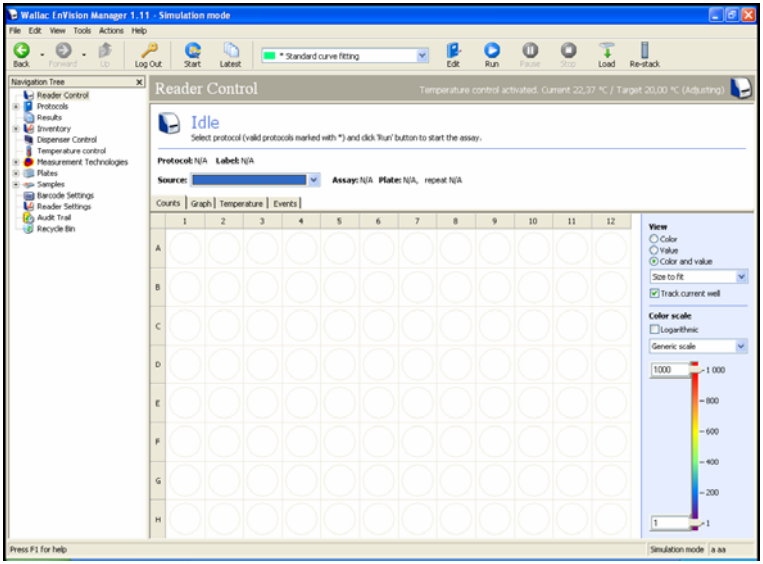
Note! If there are features described in this manual which are not in your software, it means that the feature is an option which is not installed in your instrument. Please consult the Order guide for a list of options which can be installed by a service person.

Introduction to the User Interface

When you boot the system, the main ***Reader control*** window appears. You can start assays by clicking the **Start** button. This begins the Assay Start wizard. You can also select from a list of ready protocols and start running them with the **Run** button.

Reader control window

This is the window used to control and view a run. The main parts are shown in the figure.



The menu title bar has six titles:

File menu
This menu allows **Exit** from the software.

Edit menu
Not used in ***Reader control***.

View menu
You can use this to go to one of the views e.g. ***Results***, etc. Keyboard shortcut can also be used and these are shown in the **View** menu. If you have a tree displayed then you can move back or forward in the tree or go up to the next highest level. You can also select whether you want the **Shortcuts bar** and/or the **Navigation Tree** to appear.

Tools menu
The **Assay start Wizard** shows you how to optimize and start running plates.

Before moving the instrument click **Prepare Instrument for Transport**. Confirm the selection by clicking **OK**.

Actions menu
The following functions are available under **Action**:

Latest Results - display the results that have been most recently obtained.

Edit protocol –edit the protocol shown in the drop-down menu on the ***Reader contro***l toolbar.

Run Assay – start running an assay using the protocol shown in the drop-down menu on the ***Reader control*** toolbar.

Pause – this is only enabled if an assay is running. You can temporarily stop the run and then continue it by clicking **Run**. Note that with on-the-fly and HTS AlphaScreen measurements, measuring continues until the end of the row before pausing.

Stop – this is only enabled if an assay is running. The run stops, any results are saved and the instrument returns to the Idle state.

Unload / Load – the function available depends on if there is a plate in the instrument to be unloaded, or if there is no plate, in which case one can be loaded.

Re-stack - this allows you to move plates from the output stack to the input stack.

Full Screen Plate – select this to see a full screen view of the counts tab plate. Press Esc or right click the mouse to close this view.

Help menu
Click this to get help on the use of the software. At any point during operation pressing the F1 key will cause the appearance of help information relevant to that particular operation.

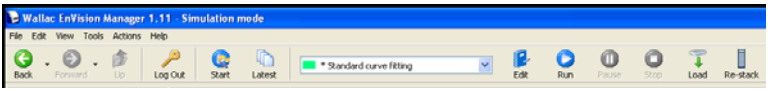
If your software malfunctions, you can select **Create Error Report** to generate a report of the software status. This will help the service person handling your case. The report is created as a zip file on your computer desktop. Copy and paste this into an email with information about the problem and send it to your service engineer.

Select **Save Screenshot To File** if you want a screenshot of your user interface at any phase in its operation. The file format is bmp. You must give the path to where the file is to be saved.

About gives information about the software version.

Reader control toolbar

Below the menu title bar is the toolbar. Some of the buttons always remain the same, others change according to the view selected.



Buttons that do not change
Three buttons, **Back**, **Forward** and **Up**, allow you to navigate in the tree structure of the Navigation Tree. The same commands are found in the **View** menu.

You can also view latest results by clicking the **Latest** button at the top of the window.

The **Logout** button appears if you have the Enhanced Security option.

The **Start** button allows you to optimize and run plates. The **Assay Start Wizard** guides you through these steps.

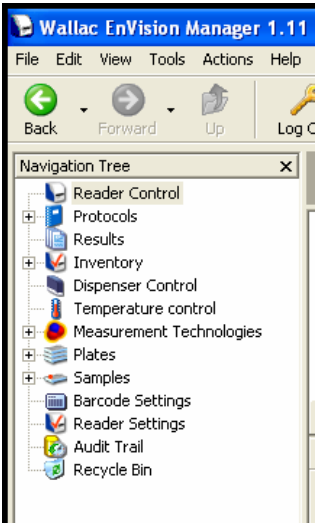
Buttons that change
The buttons here depend on the view selected. In the ***Reader control*** view the buttons enabled are **Edit**, **Run** and **Load**.**Pause**, **Stop** and **Re-stack** are disabled. See the **Action** menu for a description of these functions.

The drop-down menu allows you to select the label you are going to use. Labels with a green bar and an asterisk (*) in front are valid labels i.e. all the necessary modules and components are installed..

Configuring your user interface

The **View** menu allows you to select whether you want the **Shortcuts bar** and/or the **Navigation Tree** or neither to appear.

Navigation Tree



This gives you access to all of the functions of the workstation. There are various folders e.g. ***Reader Control***, ***Results*** etc. most of which have sub-folders.

If you are in a sub-folder, you can move to the next higher level by clicking the **Up** button.

Click the **Back** button to go to the previous location in the tree. If you have gone back, you can go forward by clicking the **Forward** button. Clicking the arrows beside one of these buttons allows you to see the possible locations in the tree and to jump directly to the one you want.

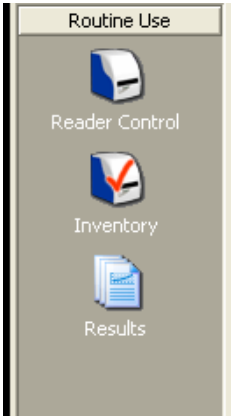
Clicking the right mouse button leads to a menu:

Open –opens the selected item

New shortcut – makes a short cut of the selected item

Hide Navigation Tree – closes the navigation tree. Select it from the **View** menu to reopen it.

Shortcut Bar



It may be that the **Navigation Tree** contains a lot more items than you normally want to use. E.g. if you just work with ***Reader control*** and ***Results***, you may find it more convenient to use the shortcut bar and set it to only show these two items.

To edit the contents of the **Shortcut Bar**, first move the functions from the **Navigation Tree** to the **Shortcut Ba**r by dragging them (or selecting a function in the **Navigation Tree**, clicking the right mouse button and selecting **New Shortcut**). When you have the items you want in the **Shortcut Bar**, you can use the **View** menu (or

right mouse button menu) to close the **Navigation Tree**. You can then just use the icons in the **Shortcut Bar** until such time as you need some additional function from the **Navigation Tree**.

To delete a shortcut from the **Shortcut Bar**, right click on a shortcut and select **Delete From Shortcut Bar**.

With the right mouse button menu, you can also **Reset Shortcut Bar** so that it goes to the default configuration (***Reader Control*** and ***Results***), or **Hide Shortcut Bar**.

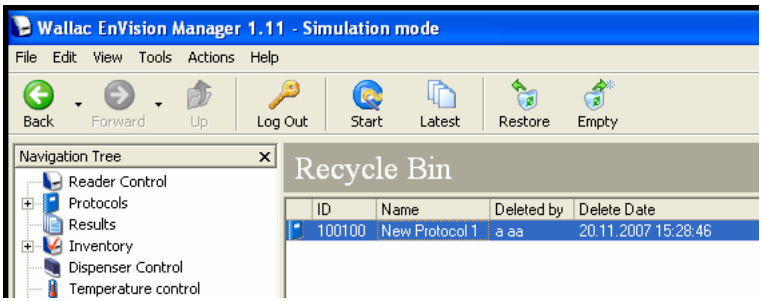
You can also create other shortcut **Groups**, e.g. you could have one group with shortcuts for running and viewing results and a second group connected with protocol editing. You can freely **Delete Group** or **Rename Group** (except if there is only one group, then it cannot be deleted). You can select whether you have **Large icons** or **Small icons** in a group depending on what appearance you like.

All these operations are possible with the menu under the right mouse button.

Note! The **Advanced Use** group contains all the main icons from the **Navigation Tree**.

Recycle Bin

The ***Recycle Bin*** folder stores deleted results, protocols, plates etc.



To restore a deleted item, select it and click the **Restore** button on the toolbar. Alternatively you can click the right mouse button. A **Restore** button will appear. Click this. The item will disappear from the ***Recycle Bin*** and will be restored to the location from which it had been deleted.

Note! When a protocol is deleted, all results measured with that protocol are also deleted. When you restore a deleted protocol, any results that were deleted with it are not restored.

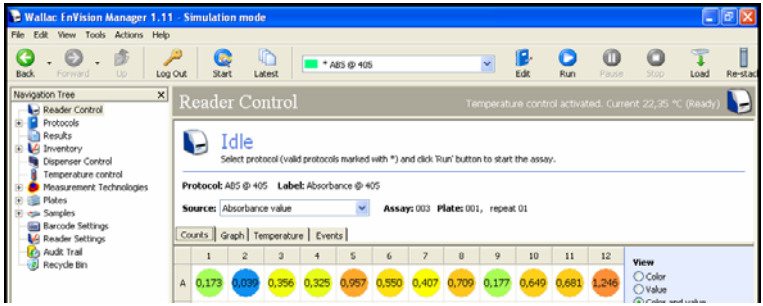
You can also permanently delete all items from the ***Recycle Bin***. Click the **Empty** button on the toolbar. Confirm that you want to permanently delete all items by clicking **OK** or click **Cancel** to avoid deleting the contents of the ***Recycle Bin***.

Note! If you are using Enhanced Security, there are restrictions on deletion and restoring. See the Enhanced Security Administrator manual for more details.

Tabs in the Reader control window

In the ***Reader control*** window there are four tabs: **Counts, Graph, Temperature** or **Events**.

Reader control window - Counts


Wallac EnVision Manager 1.11 - Simulation mode
File Edit View Tools Actions Help
Back Forward Up Log Out Start Latest * ABS @ 405 Edit Run Pause Stop Load Re-stack
Navigation Tree
Reader Control
Protocols
Results
Inventory
Dispenser Control
Temperature control
Measurement Technologies
Plates
Samples
Barcode Settings
Reader Settings
Audit Trail
Recycle Bin
Reader Control
Temperature control activated. Current 22,35 °C (Ready)
Idle
Select protocol (valid protocols marked with *) and click 'Run' button to start the assay.
Protocol: ABS @ 405 Label: Absorbance @ 405
Source: Absorbance value Assay: 003 Plate: 001, repeat 01
Counts Graph Temperature Events
1 2 3 4 5 6 7 8 9 10 11 12
A 0,173 0,039 0,356 0,325 0,957 0,550 0,407 0,709 0,177 0,649 0,681 1,246
View
Color
Value

Note! If results have been obtained after clicking **Run** the intensity of the signal from each well is shown by how the well is colored. If it is gray, the sample has saturated the detector.

At the top of the window is information about the measurement. The status of the instrument is shown and information is given on what you can do. E.g. if it is Idle you can start the run by clicking **Run**.

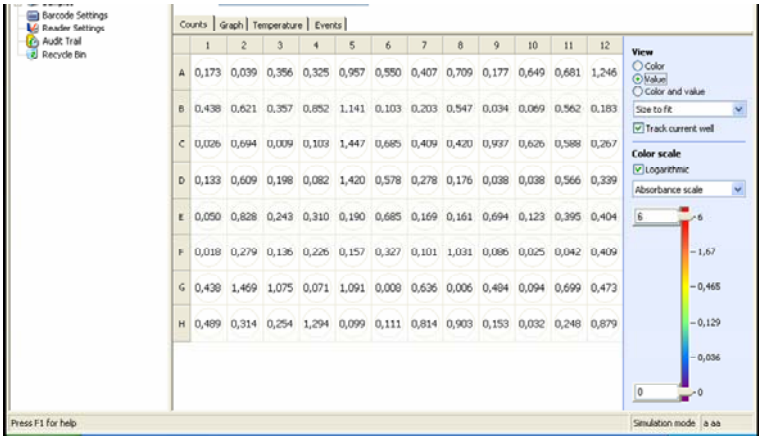
The side-bar on the right of the window gives you the following options.

View

Three option buttons allow you to select how you want results displayed. The options are: by **Color**, **Value** (numerically) or **Color and value**.

If you select **Color** then the stronger the signal, the more red the color is. The weaker the signal the more blue it is.

If you select **Value** you will see the actual counts or absorbance values on a white background.



If you select **Color and value,** then they will appear on a colored background where the color shows the signal strength.

There is a drop-down list where you can select the size of the plate display. You will need this for numerical values and higher density plates where the values are hard to read. Magnification can be up to 300%. Select **Size to fit** if you

want the software to determine the optimum size for seeing the whole plate.

Note! Press Ctrl F to see a full screen view of the plate. Press Esc or right click the mouse to close this view.

Track current well checkbox – select this if you want the current well to be always visible during a live display. This is useful when the plate is magnified and only some of the wells appear in the live display.

Color scale
Logarithmic – if this checkbox is not selected then the scale for the signal intensity is linear.

The range for the scale is selected from the drop-down list box. If no label has been selected then this will be a **Generic** range. If the label is selected then there is a selection of scales to choose from. These go from **Extra low** (1- 1000) to **UltraHigh** (1 to 100 000 000).

The default range for most labels is **UltraHigh** and **linear** scale.

For fluorescence polarization it is **Extra low** and **linear** scale.

For absorbance the scale is **0 – 6** and **logarithmic**.

The scale that is selected is then used the next time the label is measured.

There is also a color scale with sliders. You can move the sliders using the mouse.

The numbers by the sliders depend on the scale range chosen and the position of the slider within that range. Any results that are smaller than the lower slider will appear in e.g. violet. Any results higher than the upper slider will appear in e.g. red.

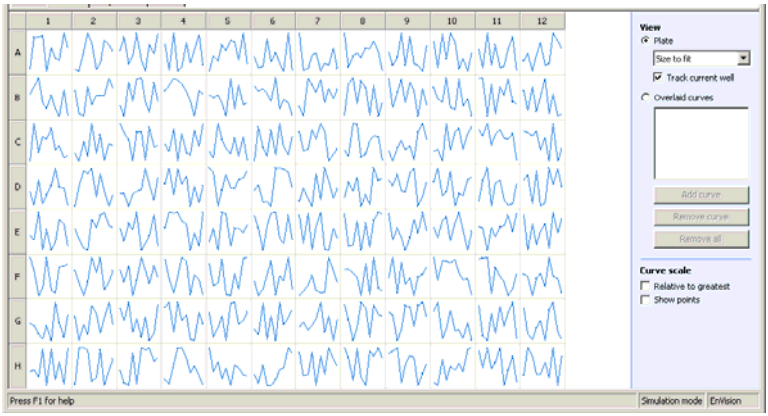
You can define the colors in ***Reader settings***.

Results between the values of the two sliders will appear in a color that corresponds to the value.

It is also possible to manually change the range by typing a value in the box and then pressing Enter.

Note! The type and number of colors used can be changed in ***Reader settings***.

Reader control window - Graph



Select this view if you are doing Plate or Assay repeats, Kinetic measurement or Dispense Measurement.

The side-bar on the right of the window gives you the following options.

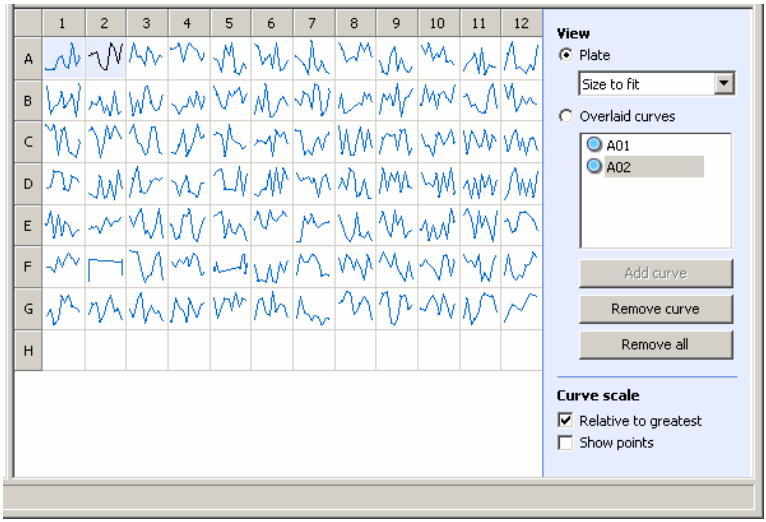
View
Plate – select this to see a picture of the plate. For each well you will see a curve showing how the signal changes with time.

There is a drop-down list where you can select the size of the plate display. You will need this for numerical values and higher density plates where the values are hard to read. Magnification can be up to 300%. Select **Size to fit** if you want the software to determine the optimum size to see the whole plate.

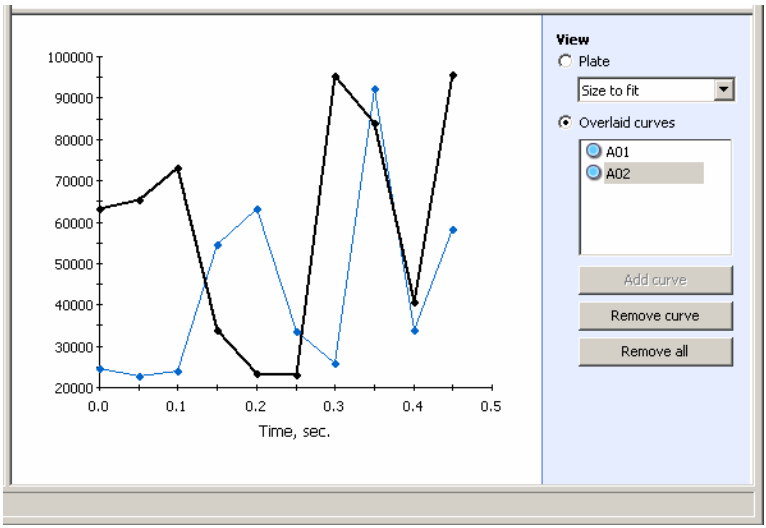
Note! Press Ctrl F to see a full screen view of the plate. Press Esc or right click the mouse to close this view.

Track current well checkbox – select this if you want the current well to be visible during a live display. This is useful when the plate is magnified and only some of the wells appear on screen.

Overlaid curves – before you select this, click the cursor on the wells you are interested in. Each time you have selected a well, click the **Add curve** button (you can also double click the well). The well coordinates will be added to the list under the **Overlaid curves** button.



When you have selected all the curves you want, click the **Overlaid curves** button. The selected curves will be displayed with measured values plotted against time.



To remove a curve from the display, select it from the **Overlaid curves** list and click **Remove curve**.

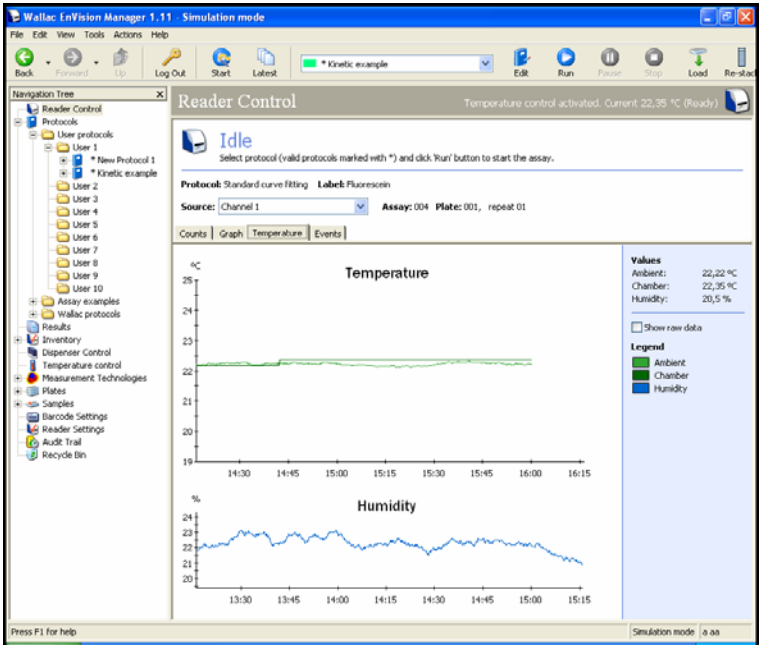
Click **Remove all** if you want to get rid of all curves from the **Overlaid curves** list.

Curve scale
These check boxes are only visible if the **Plate** option is selected.

Relative to greatest –all the curves are scaled relative to the greatest value of all the curves on the plate. This enables you to compare all of the curves but it may hide the variation in values for each curve if there is a large difference between curves. If this check box is not selected, then each curve is scaled independently. This way you get the greatest range of values for each curve but you cannot compare the size of curves with each other directly.

Show points – select this if you want the actual points to be visible. It requires adequate magnification to see the points.

Reader control window - Temperature



Temperature and humidity values are shown in this chart. These values are the temperature inside and outside the instrument and the humidity inside the instrument.

Consistent AlphaScreen measurements require a stable temperature. This is ensured by the cold plate which is in close proximity to the assay plate. You can check from this chart that the temperature has remained stable during the time of your measurements. See ***Temperature control*** in the Reference manual for how to set the temperature for AlphaScreen.

The temperature control system allows you to raise and stabilize the temperature inside the instrument for cell-based and enzyme measurements which require e.g. 37 °C. See ***Temperature control*** in the Reference manual for how to adjust the temperature control.

The side-bar on the right of the window gives you the following options.

Values
These are the current temperature around the instrument (Ambient) and in the instrument at the position of the plate (Chamber). The humidity in the instrument is also given.

Show raw data – click this to see more detailed temperature information from:

- the sensor inside the instrument at the position where air is sucked out of the instrument (right back corner at the conveyor level). This is a little above ambient.

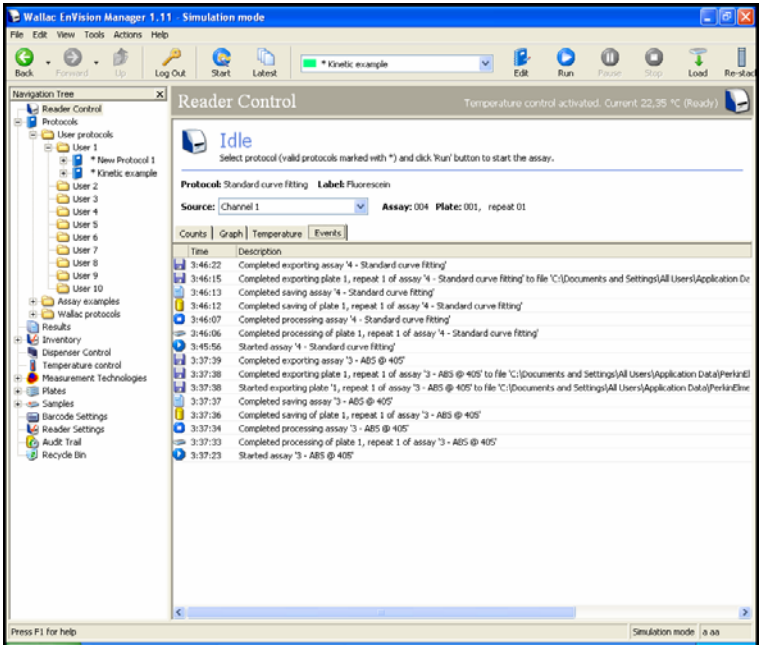
- the upper sensor above the plate

- the lower sensor below the plate conveyor.

The chamber temperature is obtained from the upper sensor unless the required temperature is set. In this case the chamber temperature is the average of the upper and lower sensors.

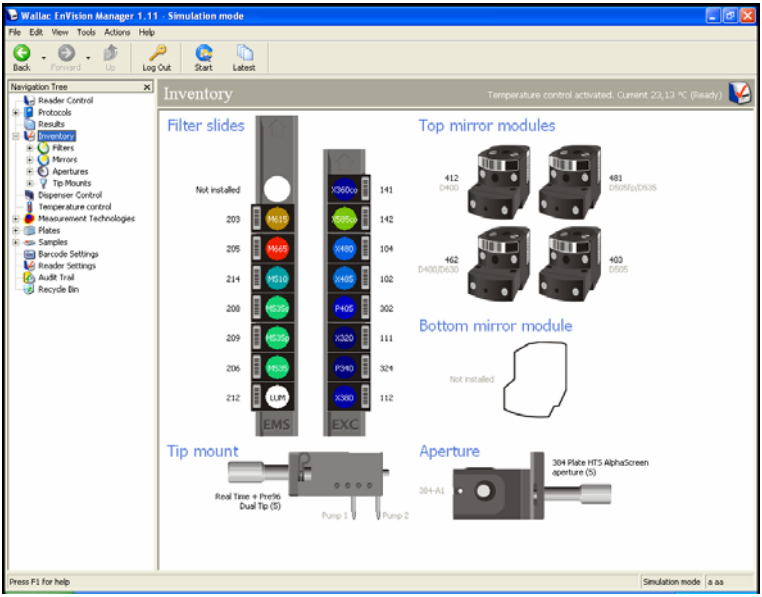
Legend
This identifies the different curves on the temperature and humidity graphs.

Reader control window - Events


Wallac EnVision Manager 1.11 - Simulation mode
File Edit View Tools Actions Help
Back Forward Up Log Out Start Latest
* Kinetic example
Edit Run Pause Stop Load Re-stack
Navigation Tree
Reader Control
Protocols
User protocols
User 1
* New Protocol 1
* Kinetic example
User 2
User 3
User 4
User 5
User 6
User 7
User 8
User 9
User 10
Assay examples
Wallac protocols
Results
Inventory
Dispenser Control
Temperature control
Measurement Technologies
Plates
Samples
Barcode Settings
Reader Settings
Audit Trail
Recycle Bin
Reader Control
Temperature control activated. Current 22,35 °C (Ready)
Idle
Select protocol (valid protocols marked with *) and click 'Run' button to start the assay.
Protocol: Standard curve fitting Label: Fluorescein
Source: Channel 1
Assay: 004 Plate: 001, repeat 01
Counts Graph Temperature Events
Time Description
3:46:22 Completed exporting assay '4 - Standard curve fitting'
3:46:15 Completed exporting plate 1, repeat 1 of assay '4 - Standard curve fitting' to file 'C:\Documents and Settings\All Users\Application Da
3:46:13 Completed saving assay '4 - Standard curve fitting'
3:46:12 Completed saving of plate 1, repeat 1 of assay '4 - Standard curve fitting'
3:46:07 Completed processing assay '4 - Standard curve fitting'
3:46:06 Completed processing of plate 1, repeat 1 of assay '4 - Standard curve fitting'
3:45:56 Started assay '4 - Standard curve fitting'
3:37:39 Completed exporting assay '3 - ABS @ 405'
3:37:38 Completed exporting plate 1, repeat 1 of assay '3 - ABS @ 405' to file 'C:\Documents and Settings\All Users\Application Data\PerkinEl
3:37:38 Started exporting plate '1, repeat 1 of assay '3 - ABS @ 405' to file 'C:\Documents and Settings\All Users\Application Data\PerkinElme
3:37:37 Completed saving assay '3 - ABS @ 405'
3:37:36 Completed saving of plate 1, repeat 1 of assay '3 - ABS @ 405'
3:37:34 Completed processing assay '3 - ABS @ 405'
3:37:33 Completed processing of plate 1, repeat 1 of assay '3 - ABS @ 405'
3:37:23 Started assay '3 - ABS @ 405'
Press F1 for help
Simulation mode

This tab shows a log of the operations performed by the instrument during a run. The log is in chronological order with the most recent operation first. The log is cleared when you shut down the Manager software.

Inventory



This window shows you visually which components are installed in your EnVision. These components are:

Filter slides – individual filters are shown in their slides
Mirror modules (top and bottom)
Tip mounts
Apertures

From this information you can determine if you have the components installed that are necessary for the operations you wish to perform. If a component is missing any protocol that needs it will not be valid i.e. there will not be an * and a green bar beside the protocol name in the protocol list.

Install any missing components and ensure you have a valid protocol before attempting to run the assay.

Refer to the reference manual for information about setting up protocols, filters, mirror modules etc.

Refer to the routine maintenance section of the Instrument manual for how to physically install components.

Chapter 2
Operating EnVision

Options for running Envision

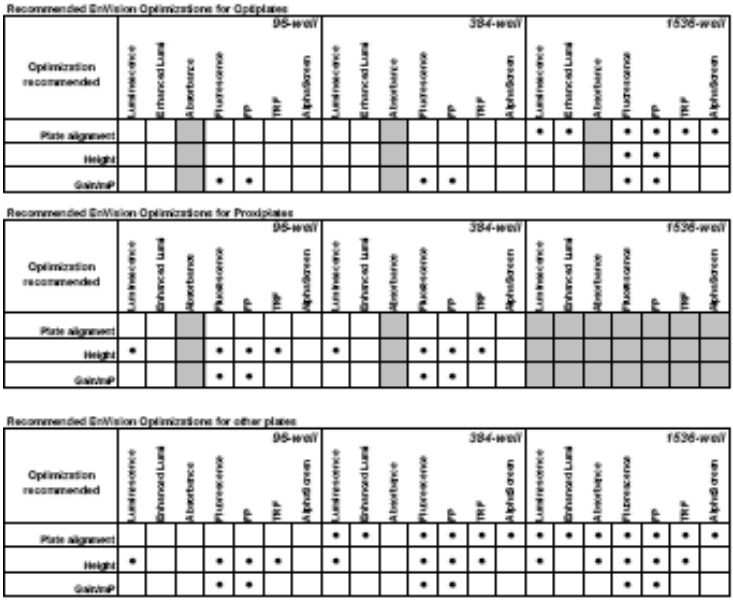
The following questions guide you to the information you need to run EnVision in the best way for you.

Do you have a protocol ready?

No	Yes
Use the **Assay Start/ Optimization Wizard** under **Tools,** or the **Start** button, to create and optimize one. Or go to the protocol editor ***Protocols*** and create a protocol, then come back to these questions. *Note!* The protocol created by the **Assay Start Wizard** has default parameters for a single plate and single label. It allows you to do an optimization and then run a single plate assay. You can edit the protocol later. To start with a protocol using parameter values different from the default ones, you must create it first before using the **Assay Start Wizard**.	The next question is then concerned with optimization.

Do you have the plate and label combination optimized?

No	Yes
Use the **Assay Start Wizard** to do the optimization. *Note!* Optimization is the procedure for fine tuning the parameters for your plate and label combination to get the very best results. For some applications you can get satisfactory results by using the default settings without optimizing. See the table. *Note!* For multiple label assays you must run the **Assay Start Wizard** for each label.	No protocol barcodes: • Use the **Assay Start Wizard** • Or select the protocol and click the **Run** button on the user interface Protocol barcodes and the barcode mode selected: • Use the **Assay Start Wizard** • Or press the START button on the instrument These options are described as well as the use of a stacker with barcodes or without barcodes. *Note!* If you later change the label or plate parameters used in optimization you must redo the optimization.



Types of optimization

Plate dimension optimization (All technologies)
This enables EnVision to determine the exact positioning of the plate being used. The position of each of the four corner wells of the plate is measured. It requires a plate with samples in each of the four corner wells using the label you intend for normal measurements. E.g. a full plate is suitable.

Measurement height optimization (All technologies)
This enables EnVision to determine the optimum focal point for measurement. A single sample is required for this. Unless you choose otherwise, the strongest sample will be identified and used. The plate is measured over a range of

vertical positions and the one giving the highest signal is determined.

Monochromator wavelength optimization (Abs)
This optimization determines the wavelength at which maximum absorbance occurs. A single sample is used. Select the lower and upper limits of the wavelength range. Select also the step length between wavelength measurements and the number of flashes. A plot of wavelength against counts is displayed and the wavelength for the lowest counts value is selected. You can adjust the result manually if required by clicking on the wavelength you want.

Monochromator wavelength optimization (FI)
This optimization determines the wavelength at which maximum emission occurs. A single sample is used. Select the lower and upper limits of the wavelength range. Select also the step length between wavelength measurements and the number of flashes. A plot of wavelength against counts is displayed and the wavelength for the highest counts value is selected. You can adjust the result manually if required by clicking on the wavelength you want.

TRF window optimization (TRF)
This optimization determines the best delay between the excitation flash and the opening of the measurement window. It also determines the duration of the window. This minimizes the background fluorescence in the measurement window while allowing the measurement to start as soon as possible and be as short as possible, thus reducing the measurement time. A low and a high sample are required. The number of flashes to be used for the

optimization needs to be selected, the default is 50. Two sets of results are displayed. The first proposes an optimum value for the delay, based on the signal to background ratio obtained. The second proposes the duration of the window. Three criteria are available for determining the optimum duration. These come from different parameter values in the equation: Y = a * S/Smax + b * [S/B]/[S/B]max.
a = 1 b = 0.5
a = 0.5 b = 1
a = 1 b = 1
Choose between these three options to get the plot that most clearly helps you determine the window you want to use. You can adjust the delay and window width manually if required.

Detector gain optimization (FP and FI)
This optimization determines the optimum gain for the detector. A single sample is required. This sample should represent the strongest intensity in the assay. Unless you choose otherwise, the strongest sample will be identified and used in the case of fluorescence intensity. For G-factor optimization in fluorescence polarization a low binding or free fluorophore sample is needed. The optimum gain is the one that is the maximum possible without saturating the detector for high signal samples.

Z' optimization (All technologies)
The optimization determines how well the sample results are separated from the background when the scatter of results is taken into account. Six high samples and six lows samples are needed. You can select the target Z' factor, the default is 0.6. In the results, the minimum number of

flashes to achieve the Z' target is selected. You can adjust this flash number manually if required.

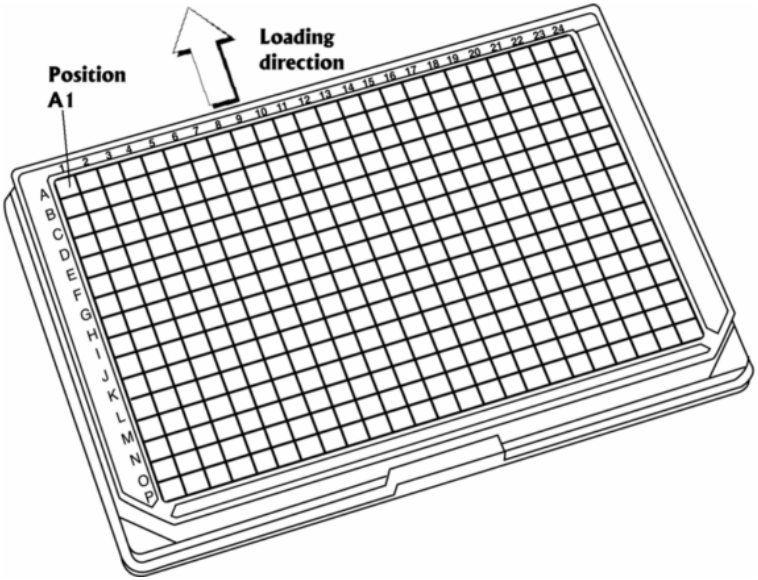
Flatfield correction
This corrects for variation in the signal across a plate due to e.g. plate curvature. In such a case the signal at the edges may be less than at the center due to the difference in distance from the detector. Identical samples need to be used in each well of the plate. The system determines the differences between each part of the plate. The correction factors calculated are applied to subsequent plate measurements. Since the position of samples for this correction cannot be combined with the positions for other optimizations, this correction has to be performed separately.

Crosstalk correction optimization (special luminescence)
This allows the glow crosstalk to be measured and corrected for. A single normal sample and a blank are used for the glow crosstalk measurement. Additional blanks are used to obtain an average blank value. See Special luminescence for more information about this optimization. An optimization plate must be used.

Crosstalk correction optimization (AlphaScreen)
This allows three types of crosstalk to be measured and corrected for. These are: glow, afterglow and bleaching. In order to measure these different types of crosstalk several configurations of samples are needed on the plate. The wizard shows what is necessary. See " AlphaScreen " for more information about this optimization. An optimization plate with a specified plate map must be used.

Plate orientation

The figure shows how a plate is to be orientated when loading it manually into the instrument. The same also applies when loading the plate to a magazine of the stacker. The A1 position must be in the left corner furthest from you i.e. the side that enters the instrument first.



Note! If you are using strip plates, make sure the strips are not sticking up at all but are properly in position, otherwise the plate may jam in the instrument. Results will not be correct if strips are not properly in position.

If you are using barcodes, these can be attached to the plate on the front or back (long) side or right (short) side, depending on which is selected in ***Reader Settings***.

Note! Correct plate labeling, sample preparation and sample order on a plate are critical factors in obtaining correct results.

Using the Assay Start Wizard

To get optimum results with EnVision you should optimize the measurement technology and plate combination you are going to use in your measurements.

Assay Start Wizard

The **Assay Start Wizard** guides you through the process of optimization. If you have an optimized plate and technology combination you can still use the **Assay Start Wizard** to start your run.

Use of an optimization plate

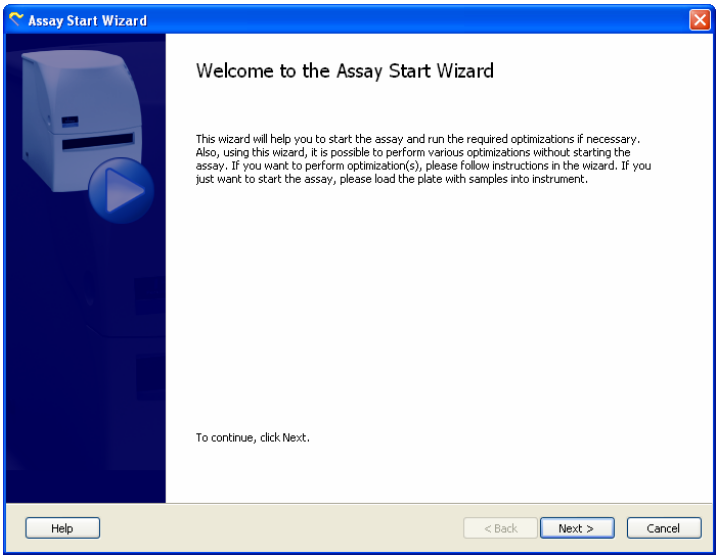
For most technologies you do not need a special optimization plate, although you can use one. A normal full assay plate is sufficient. When you have done an optimization with an assay plate you can start to run it directly from the **Assay Start Wizard**.

Note! Special luminescence and AlphaScreen require an optimization plate to be used.

Click the **Start** button to start the assay start wizard.

Steps in running the Assay Start Wizard

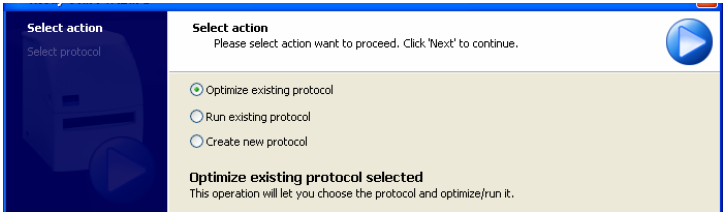
1. Welcome to the Assay Start Wizard


Assay Start Wizard
Welcome to the Assay Start Wizard
This wizard will help you to start the assay and run the required optimizations if necessary. Also, using this wizard, it is possible to perform various optimizations without starting the assay. If you want to perform optimization(s), please follow instructions in the wizard. If you just want to start the assay, please load the plate with samples into instrument.
To continue, click Next.
Help
< Back
Next >
Cancel

Click **Next**.

2. Select action

Three option buttons allow you to select your action.


Select action
Select protocol
Select action
Please select action want to proceed. Click 'Next' to continue.
Optimize existing protocol
Run existing protocol
Create new protocol
Optimize existing protocol selected
This operation will let you choose the protocol and optimize/run it.

Select the option button and click **Next**.

The table shows the different steps of the wizard depending on the option you have selected

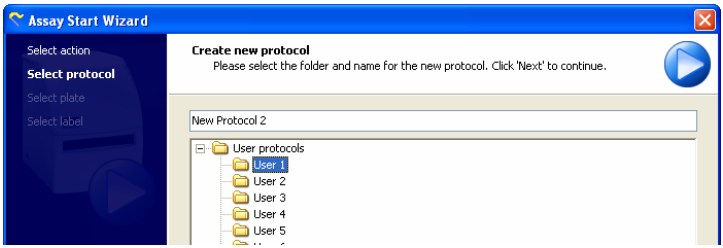
<table>
<tr><th>Create new protocol</th><th>Optimize existing protocol</th><th>Run existing protocol</th></tr>
<tr><td>Select folder for new protocol</td><td colspan="2">Select protocol</td></tr>
<tr><td>Select plate</td><td rowspan="2"></td><td rowspan="5"></td></tr>
<tr><td>Select technology</td></tr>
<tr><td colspan="2">Select optimization</td></tr>
<tr><td colspan="2">Define plate layout</td></tr>
<tr><td colspan="2">Run optimization</td></tr>
<tr><td colspan="3">Finish</td></tr>
</table>

The following explanation is based on the Create new protocol option with comments about the other options where relevant.

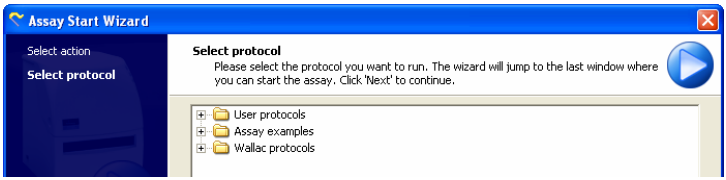
Note! The protocol created with the **Assay Start Wizard** will use default parameter settings and is for one plate only. You must use the protocol editor ***Protocols*** if you want to define the protocol parameters yourself.

3. Protocol

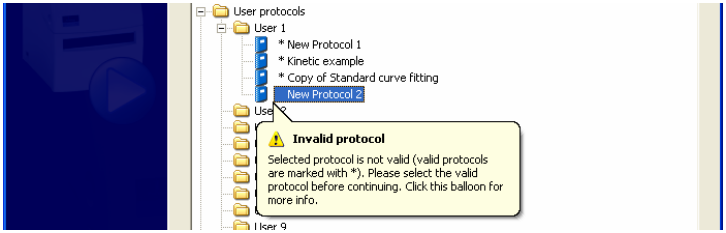
If you are creating a new protocol you will be asked to tell in which folder the protocol is to be saved. Select the folder from the tree and click **Next**.



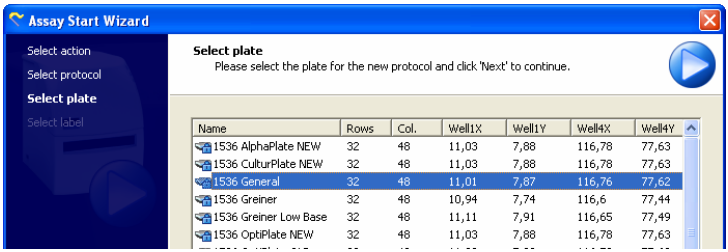
Note! If instead you are using an existing protocol for optimization or running, select it from the tree.


Assay Start Wizard
Select action
Select protocol
Select protocol
Please select the protocol you want to run. The wizard will jump to the last window where you can start the assay. Click 'Next' to continue.
User protocols
Assay examples
Wallac protocols

Note! If you select a protocol for a run but the protocol is not optimized, an error message will appear and you must optimize the protocol.

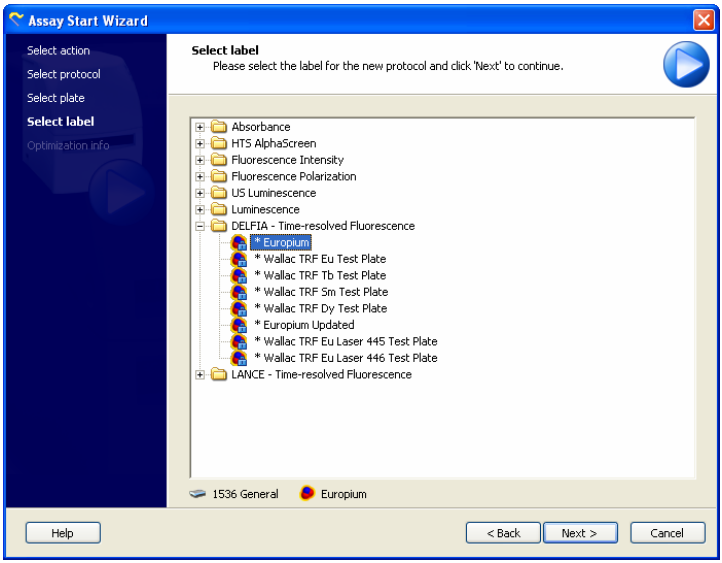

User protocols
User 1
* New Protocol 1
* Kinetic example
* Copy of Standard curve fitting
New Protocol 2
Invalid protocol
Selected protocol is not valid (valid protocols are marked with *). Please select the valid protocol before continuing. Click this balloon for more info.

4. Select plate


Assay Start Wizard
Select action
Select protocol
Select plate
Select label
Select plate
Please select the plate for the new protocol and click 'Next' to continue.
Name
Rows
Col.
Well1X
Well1Y
Well4X
Well4Y
1536 AlphaPlate NEW 32 48 11,03 7,88 116,78 77,63
1536 CulturPlate NEW 32 48 11,03 7,88 116,78 77,63
1536 General 32 48 11,01 7,87 116,76 77,62
1536 Greiner 32 48 10,94 7,74 116,6 77,44
1536 Greiner Low Base 32 48 11,11 7,91 116,65 77,49
1536 OptiPlate NEW 32 48 11,03 7,88 116,78 77,63

Select the plate to be used. The name of the plate selected will appear at the bottom of the window. Click **Next**.

5. Select technology

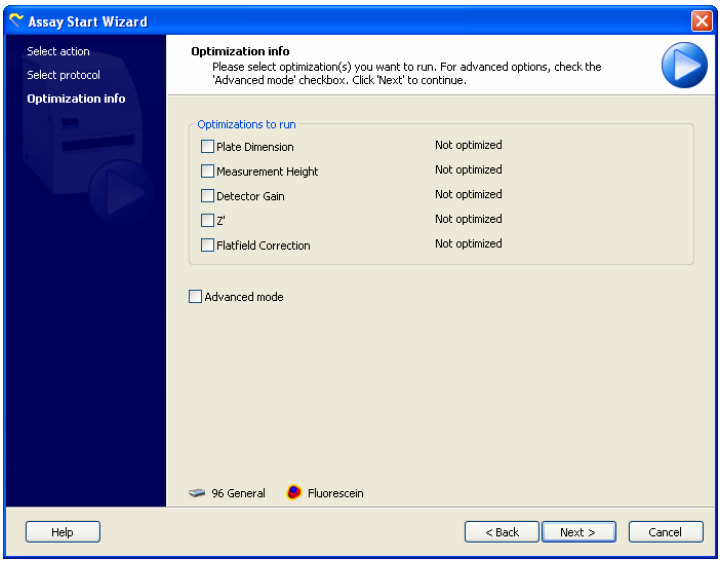


Select the technology to be used. The name selected will appear at the bottom of the window. Technologies marked with * are ready to be used for measurements. Click **Next**.

Note! If you are using a protocol with multiple technologies, you must run the **Assay Start Wizard** as many times as there are labels to optimize. When the final technology is optimized, then you can run the assay.

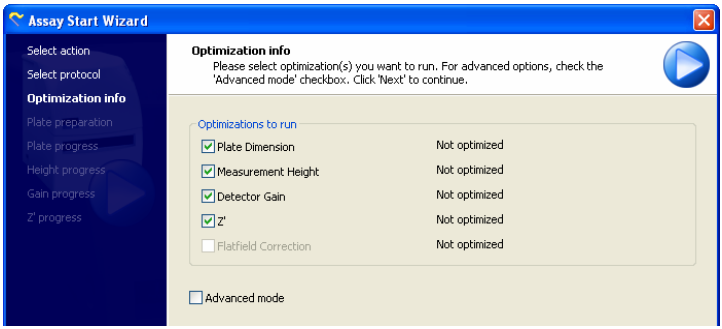
6. Optimization information

Note! This step in the wizard and the following ones are common to both the **Create new protocol** and **Optimize existing protocol** options.

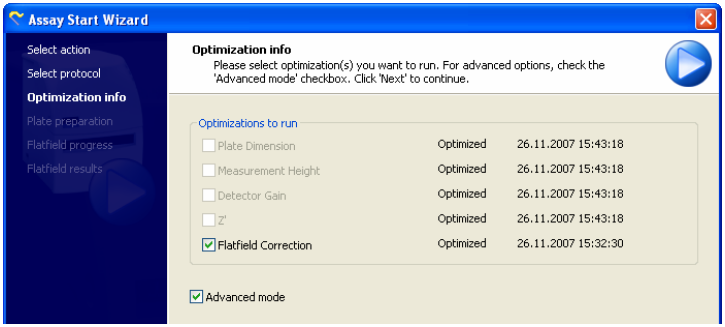


This shows you which optimizations are needed for the plate and technology combination you have chosen and if any of these optimizations have been done already.

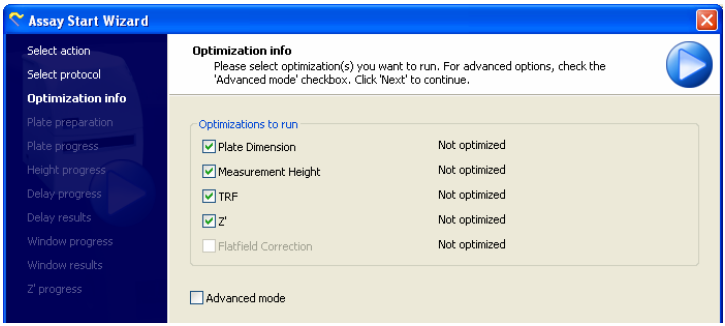
You can select any or all of the first four optimizations. The fifth, Flatfield, will be disabled because it cannot be done at the same time as any of these.



Alternatively, if you select Flatfield correction the other four will be disabled.


Assay Start Wizard
Select action
Select protocol
Optimization info
Plate preparation
Flatfield progress
Flatfield results
Optimization info
Please select optimization(s) you want to run. For advanced options, check the 'Advanced mode' checkbox. Click 'Next' to continue.
Optimizations to run
Plate Dimension Optimized 26.11.2007 15:43:18
Measurement Height Optimized 26.11.2007 15:43:18
Detector Gain Optimized 26.11.2007 15:43:18
Z' Optimized 26.11.2007 15:43:18
Flatfield Correction Optimized 26.11.2007 15:32:30
Advanced mode

Note! If the optimization involves the TRF laser technology, there will be a **TRF** optimization instead of **Detector Gain**.

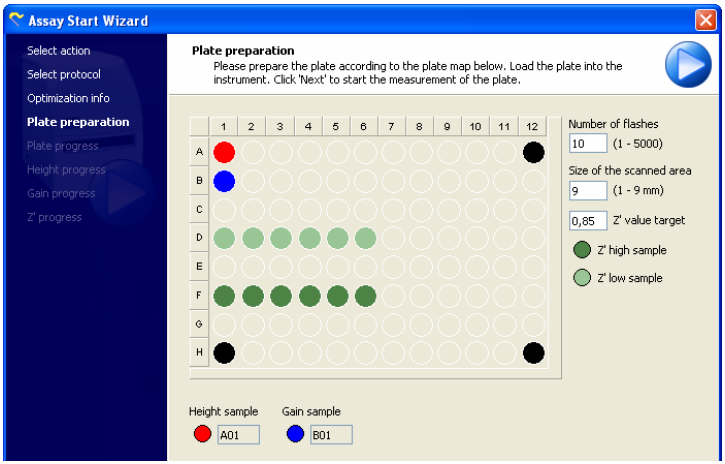


Choose the optimizations you want to be done or redone. Check boxes allow you to make this selection. If you click **Next** without selecting an optimization, the wizard will jump to the last window.

Note! Check **Advanced** only if you want to inspect or change the selections made by the software.

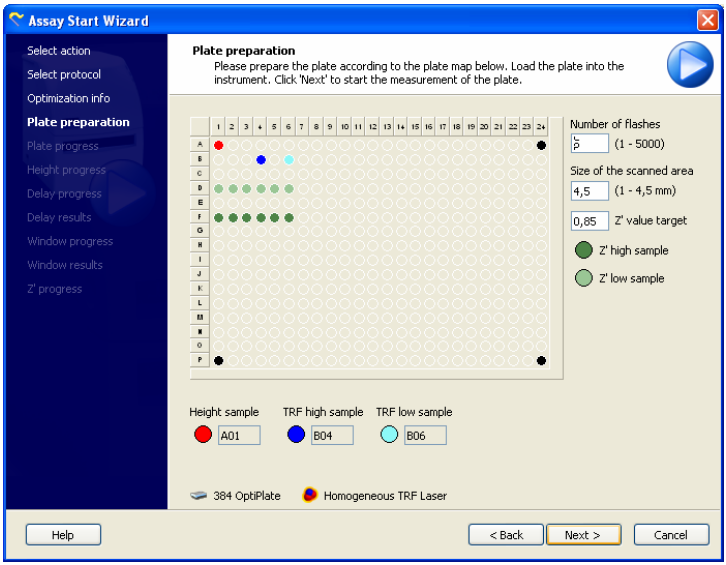
This example continues without **Advanced** being selected. See "Advanced Optimization" for examples of screenshots that appear when **Advanced** is selected. Click **Next**.

7. Plate preparation



The first example shows where samples must be placed for the optimizations selected when Detector Gain is one of the optimizations.

The second example shows where samples must be placed for the optimizations selected when TRF is one of the optimizations.

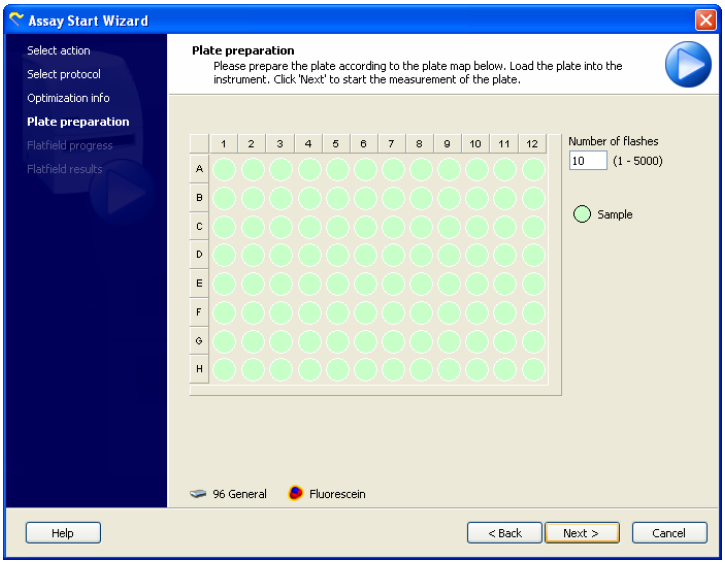


If Plate dimension optimization is selected, you must have samples at least in the corners of the plate, e.g. an assay plate with samples in every well would be suitable.

The sample for height optimization can be the same as the A1 corner well for plate optimization.

The sample for Gain optimization is a separate sample in well B1.

Z’ optimization requires high and low samples in the positions shown in the screenshot.



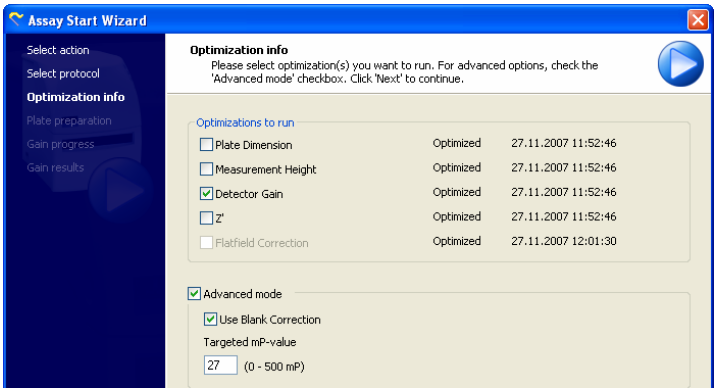
If flatfield correction is select a platemap appears showing where samples must be placed. Every well should have an identical sample in it so that the flatfield correction can be calculated.

When you have ensured that your samples are in the correct layout in the plate, load your plate and click **Next**.

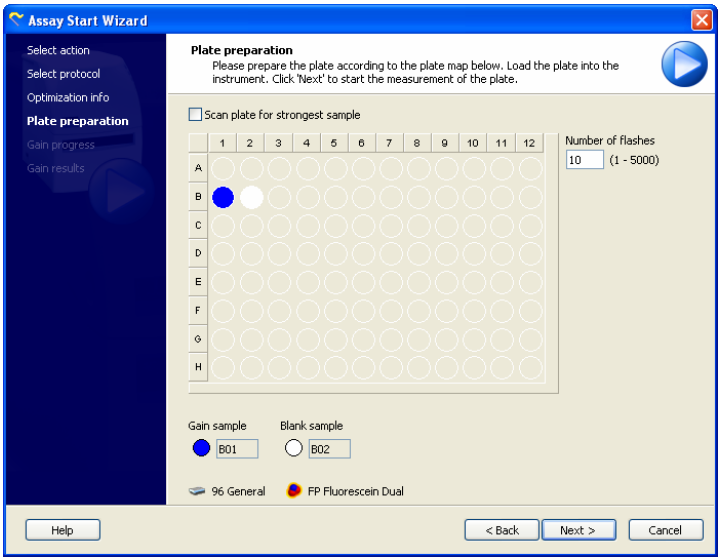
Note! In general, unless you have selected **Advanced** you should not edit the **Number of Flashes** or **Size of the Scanned Area** even though you can do this. See "Advanced Optimization" for information about these parameters.

Note! If your protocol uses fluorescence polarization, the **Advanced** check box will be selected by default. If

Detector Gain optimization is selected, you can select to use a **Blank** sample and give the **Target mP** value. This is so that you can check that the S and P signal levels are in a suitable range (200 000 - 400 000 per flash) and that they match the target mP value.

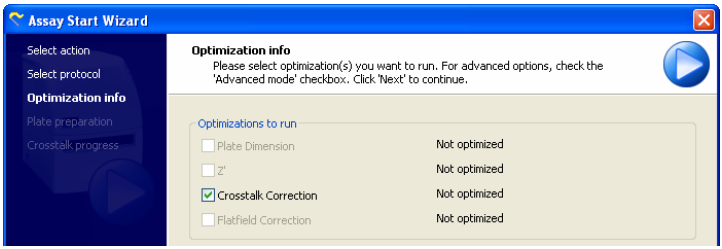


The blank sample should be put next to the Gain sample on the cassette.

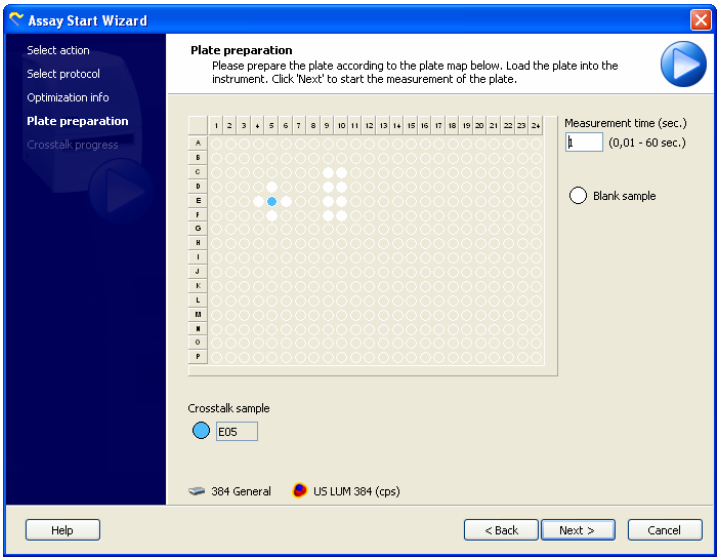


Note! For AlphaScreen and special luminescence you must use an Optimization plate with the correct arrangement of samples to do crosstalk correction.

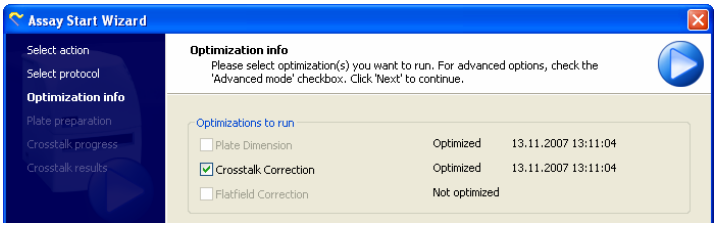
Crosstalk correction optimization (special luminescence)



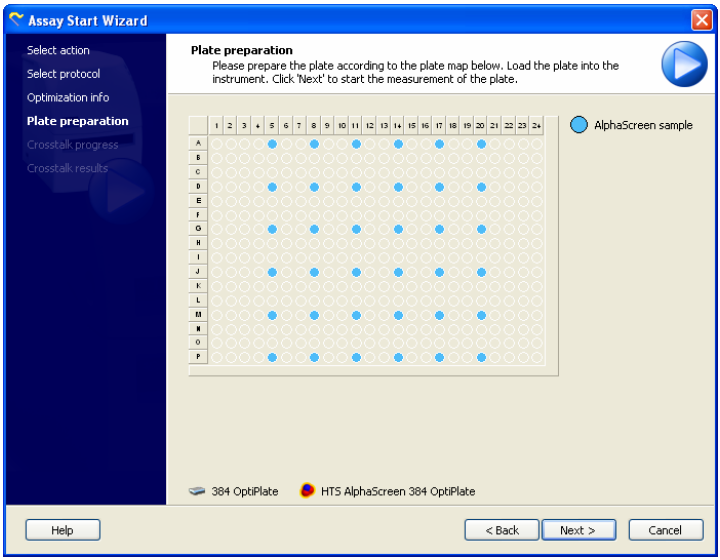
The plate map shows the correct arrangement of samples.



Crosstalk correction optimization (AlphaScreen)

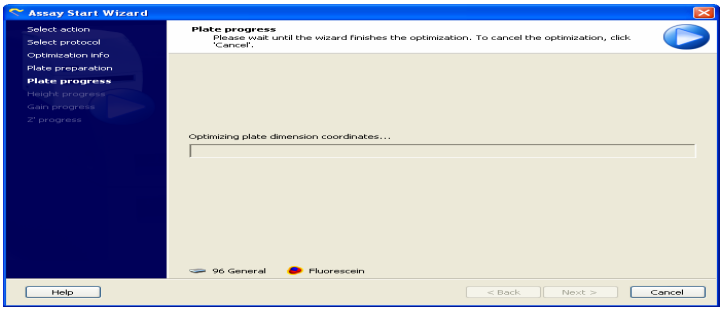


The plate map shows the correct arrangement of samples.

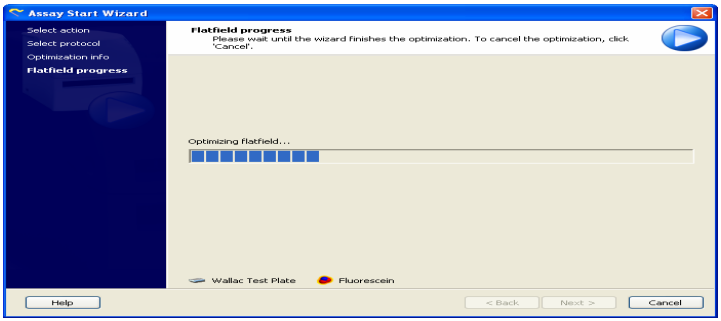


Note! The platemap for 1536 AlphaScreen is set so that samples can be transferred from 384-well plates (shown in the map) by a robotic system using 384 needles (tips).

8. Optimization progress



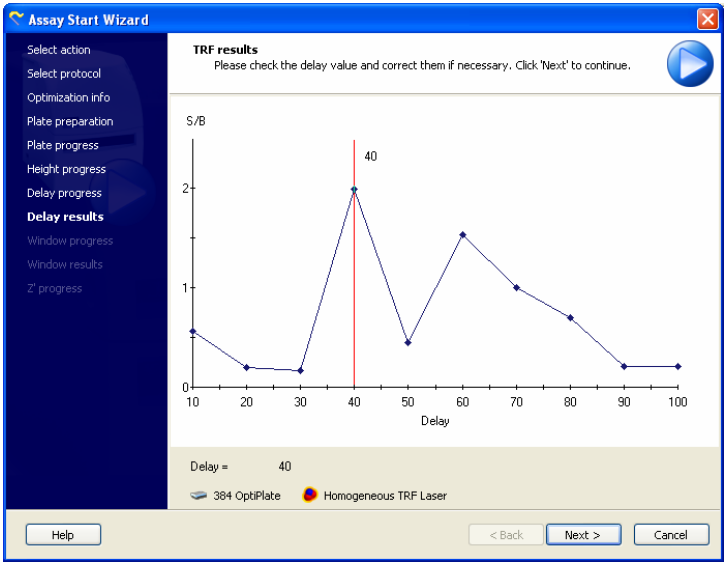
This is what you see if the optimizations are selected.



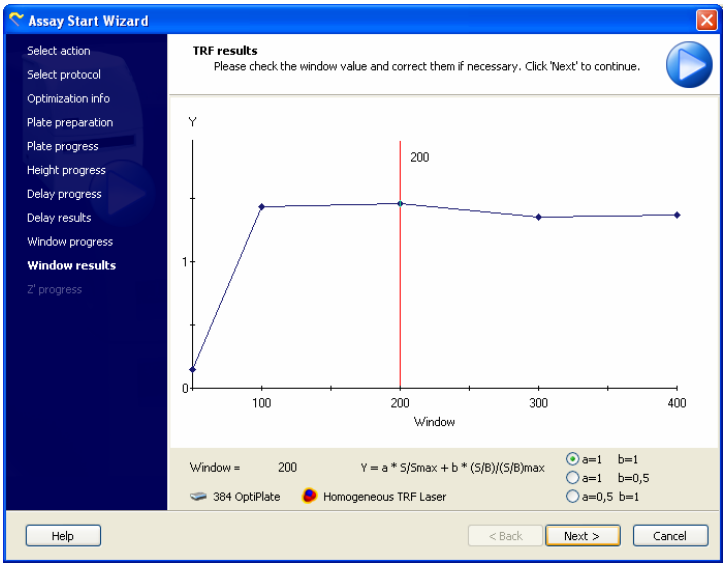
This is what you see if flatfield correction is selected.

9. Results

If TRF optimization is selected you will first be shown a window with a proposed delay.



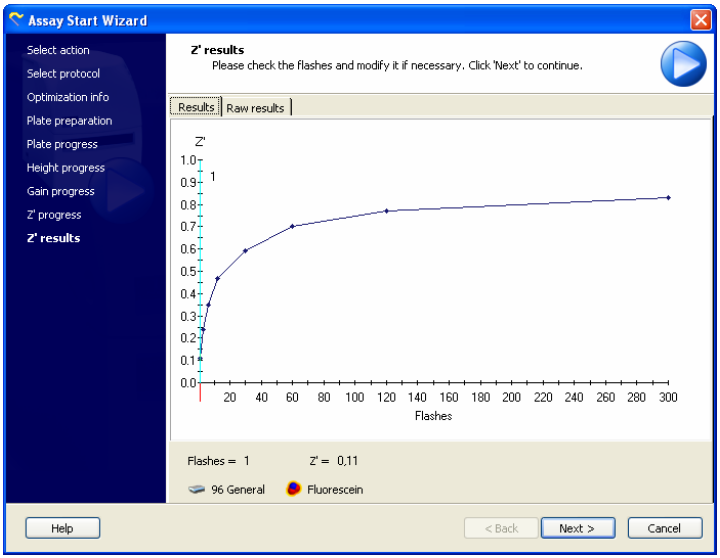
You can accept this or move the cursor to the delay you want. Then click **Next**.



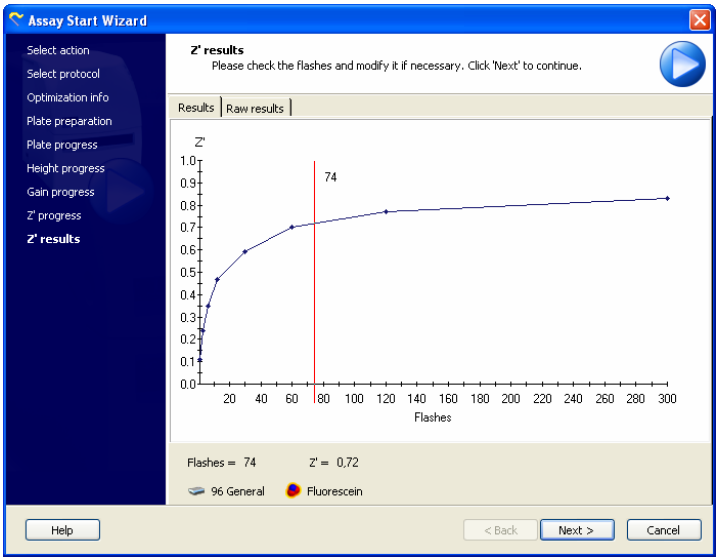
The proposed window setting will appear. Once again you can approve it or modify it by moving the cursor- Then click **Next**.

Note! These windows will not appear if TRF is not selected.

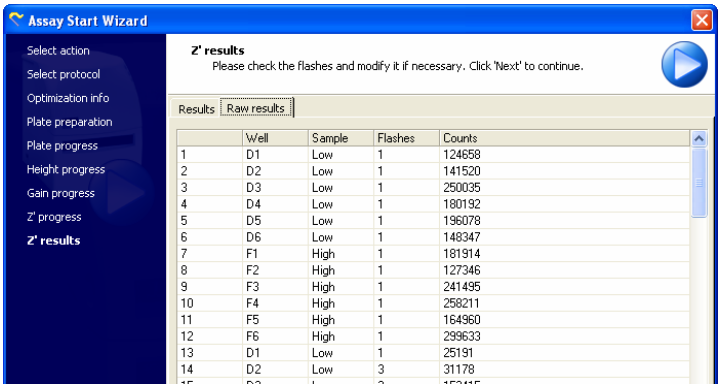
The next window only appears if Z’ is selected.



You will be shown the results of the Z' optimization and asked to check it and if necessary change the number of flashes to be used.

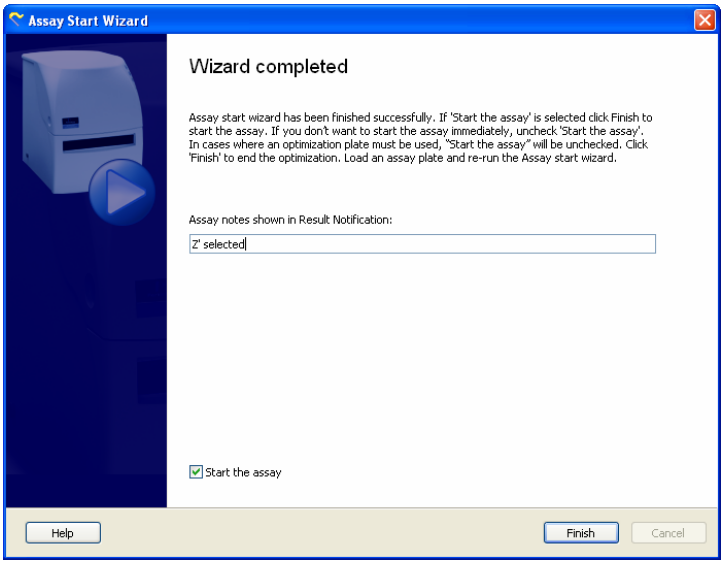


You can also see the results numerically.



When you have done this, click **Next**. The last window of the wizard will appear.

10. Wizard completed


Assay Start Wizard
Wizard completed
Assay start wizard has been finished successfully. If 'Start the assay' is selected click Finish to start the assay. If you don't want to start the assay immediately, uncheck 'Start the assay'. In cases where an optimization plate must be used, "Start the assay" will be unchecked. Click 'Finish' to end the optimization. Load an assay plate and re-run the Assay start wizard.
Assay notes shown in Result Notification:
'Z' selected
Start the assay
Help
Finish
Cancel

The **Start the Assay** check box in the last window of the wizard allows you to select if you actually want to run a plate using the optimization just done, or if you just want to save the optimization information. For optimizations where a special optimization plate is not necessary, this box will be checked by default. If an optimization plate is necessary, the box will be unchecked by default. In the latter case the window will just close when you click **Finish**.

If **Start the assay** is checked, the currently loaded plate will be run and the results displayed.

You can add a note that will be included in Result Notification.

Running an assay without the Assay Start Wizard

There are different ways of operating EnVision depending on the combination of plate loading method and barcode setting you have. Read the text describing the combination you are going to use.

Note! Before beginning any loading make sure your plates are orientated properly.

Running an assay (no protocol barcodes, no stacker)

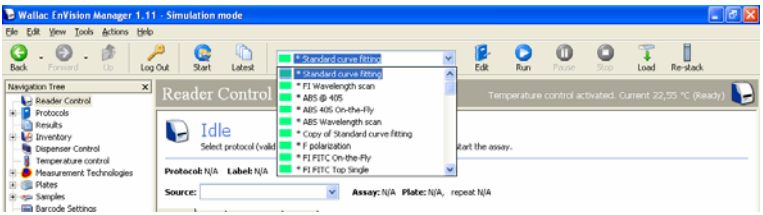
1. If the plate carrier is in the instrument, press the LOAD button or click the **Unload** button on the toolbar.

The plate carrier will come out.

Note! The software button shows **Unload** if the plate carrier is in the instrument and **Load** if it is out.

2. Load your first plate. Make sure the plate is orientated correctly with the A1 position in the left hand corner furthest from you.

3. Select the protocol you want to use from the drop-down list of protocols.

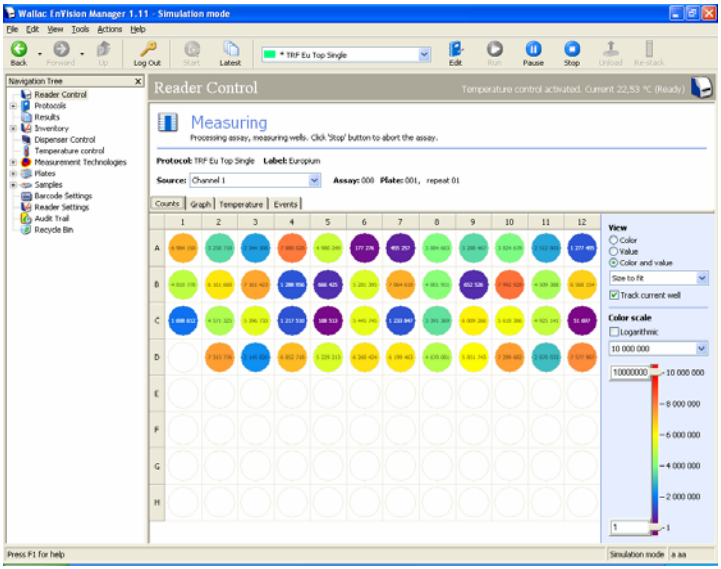


4. Use the mouse to click the **Run** button on the user interface. The plate carrier with the plate will go into the instrument and measurement will begin.

   Note! If START is pressed on the instrument, the last run protocol is repeated, not the one selected from the drop-down list.

   During a run you can click either the **Pause** or **Stop** buttons on the toolbar if you need to pause or stop the measurement.

   Pressing the STOP button on the instrument has the same effect.



5. You can follow the measurement by means of the plate map with colors indicating the intensity of the signal

from each well. At the top of the window there is information about the status of the measurement. Depending on the measurement, you can select the Source of the signal (e.g. which channel is displayed) from the drop-down list (fluorescence polarization also has the mP value calculated).

6. When the plate has been measured, the plate carrier will come out and you can remove the plate. If more than one plate has been defined in the protocol then load the next one.

7. Press the START button on the instrument or click **Run** on the user interface.

Continue this procedure until the run stops. It will stop automatically when the number of plates defined in the protocol has been reached.

If **Number of Plates** is **Unlimited**, you have to press STOP or click **Stop** to end the protocol.

Running an assay with protocol barcodes (no stacker)

Note! Make sure you set the barcode mode in ***Reader settings*** under the **Barcode** tab. You must select there the barcode to be read and then either select **Define the protocol using** or **Split barcode** for the protocol. You must also have defined the links between the barcodes and protocols as described in ***Barcode settings***.

Note! In Barcode mode the **Run** button is not enabled.

1. If the plate carrier is in the instrument, press the LOAD button or click the **Unload** button on the toolbar.

The plate carrier will come out.

Note! The software button shows **Unload** if the plate carrier is in the instrument and **Load** if it is out.

2. Load your first plate. Make sure the plate is orientated correctly with the A1 position in the left hand corner furthest from you. The plate should have a barcode on it to select the protocol to be used to count it.

3. Press the START button on the instrument. The plate carrier with the plate will go in to the instrument and measurement will begin. During a run you can click either the **Pause** or **Stop** buttons if you need to pause or stop the measurement. Pressing the STOP button on the instrument has the same effect.

4. You can follow the measurement by means of the plate map with colors indicating the intensity of the signal from each well. At the top of the window there is information about the status of the measurement.

5. When the plate has been measured, the plate carrier will come out and you can remove the plate. If more than one plate has been defined in the protocol then load the next one.

6. Press the START button on the instrument.

Continue this procedure until the run stops. It will stop automatically when the number of plates defined in the protocol has been reached.

Note! If plates after the first one have no barcodes they will be counted with the protocol defined by the first plate barcode. If they have their own barcode then the protocol defined by that barcode will be used to count the plate.

Running an assay with a stacker (no protocol barcodes)

1. Load the stacker. In ***Reader settings*** **Stacker** you can select which stacker is for loading plates and which for unloading. Make sure the plates are orientated correctly with the A1 position in the left hand corner furthest from you.

   Note! Check that the handles of both magazines are down during a run so that the plates can move upwards without hindrance.

2. Select the protocol you want from the drop-down list of protocols.

3. Use the mouse to click the **Run** button on the user interface.

   During a run you can click either the **Pause** or **Stop** buttons if you need to pause or stop the measurement. Pressing the STOP button on the instrument has the same effect.

   Note! If you want to run assay repeats with an undefined number of plates in the magazine (i.e. select **Unlimited**) then select "End the assay when stacker is out of plates" in ***Reader settings*** **Stacker**.

4. You can follow the measurement by means of the plate map with colors indicating the intensity of the signal from each well. At the top of the window there is information about the status of the measurement.

All the plates in the stacker will be measured if you have selected the **Unlimited** number of plates mode in ***Protocols***. Otherwise the run will stop when the selected number of plates has been counted.

Running an assay with a stacker and protocol barcodes

Note! Make sure you set the barcode mode and have defined the links between the barcodes and protocols as described in ***Barcode settings***.

1. Load the magazines into the stacker. One of the magazines should have plates loaded in it and the other should be empty. In ***Reader settings*** **Stacker** you can select which stacker is for loading plates and which for unloading. Make sure the plates are orientated correctly with the A1 position in the left hand corner furthest from you. At least the first plate should have a barcode on it to select the protocol to be used to count it.

Note! Check that the handles of both magazines are down during a run so that the plates can move upwards without hindrance.

2. Press the START button on the instrument. The plate carrier with the plate will go in to the instrument and measurement will begin.

   During a run you can click either the **Pause** or **Stop** buttons if you need to pause or stop the measurement. Pressing the STOP button on the instrument has the same effect.

3. You can follow the measurement by means of the plate map with colors indicating the intensity of the signal from each well. At the top of the window there is information about the status of the measurement.

All the plates in the stacker will be measured if you have selected the **Unlimited** for the Number of plates parameter in ***Protocols***. Otherwise the run will stop when the selected number of plates has been counted.

Note! If plates after the first one have no barcodes they will be counted with the protocol defined by the first plate barcode. If they have their own barcode then the protocol defined by that barcode will be used to count the plate.

Buttons on EnVision

These buttons have the following functions:

START button

This button will light up when you press it and remain lit as long as a measurement is continuing. If the plate carrier is out when you press the START button, the carrier will go into the instrument and the measurement will start with the last protocol that has been run. If the system has just been switched on there is no previous run and no measurement is done.

When a plate has been measured, the plate carrier will come out. If other plates have been defined in the protocol, the START light will be on. If all the plates required by the protocol have been measured, the START light will be off.

STOP button

The light on this button will be lit as long as no measurement is occurring.

If there is a run occurring and you press this button the run will stop and the plate carrier will come out and the STOP light will come on.

The raw results of the samples measured up to the time when the STOP button was pressed will be valid.

LOAD button

Press the LOAD button to bring out the plate carrier so that you can load a plate. When you have loaded the plate, either press the LOAD button again or press START to move the plate carrier and plate into the instrument. The LOAD button will light up during the time the loading or unloading is occurring, otherwise it will be off.

Order of Dual label measurement in single detector instruments

In the case of single detector instruments, a different procedure is followed for dual label measurements than for instruments with two detectors. This is to decrease the

number of times the emission slide moves and to speed up the measurement of dual labels. The order of measurements is:

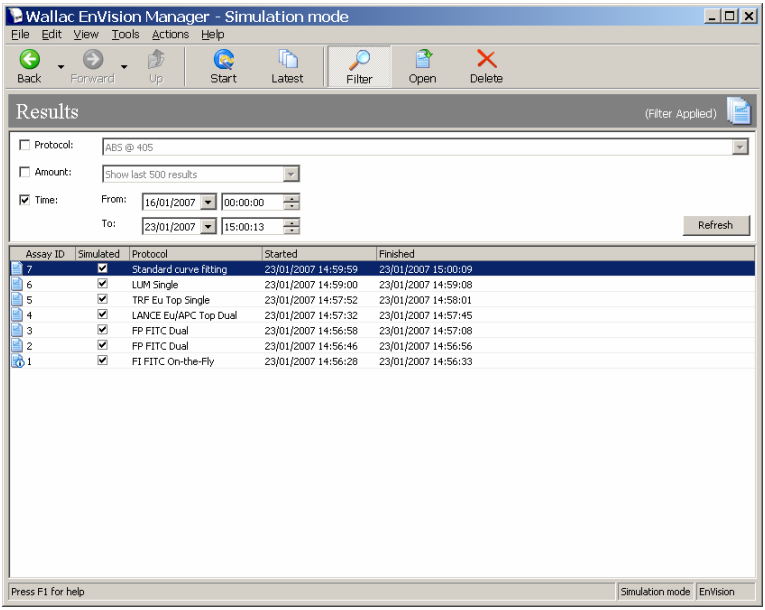
- Well 1 emission slide position 2 followed by emission slide position 1
- Well 2 emission slide position 1 followed by emission slide position 2
- Well 3 emission slide position 2 followed by emission slide position 1
- etc.

Note! This is valid for Dual excitation labels which are always measured with one detector (e.g. two excitation filters and one emission filter as in the case of the Fura-2 label shown with the Dispenser option in simulation mode).

Chapter 3
Result viewing

Result viewing

To see results click on ***Results*** in the Navigation Tree. A list of assays and dates will appear.


Wallac EnVision Manager - Simulation mode
File Edit View Tools Actions Help
Back Forward Up Start Latest Filter Open Delete
Results
(Filter Applied)
Protocol: ABS @ 405
Amount: Show last 500 results
Time: From: 16/01/2007 00:00:00
To: 23/01/2007 15:00:13
Refresh
Assay ID Simulated Protocol Started Finished
7 Standard curve fitting 23/01/2007 14:59:59 23/01/2007 15:00:09
6 LUM Single 23/01/2007 14:59:00 23/01/2007 14:59:08
5 TRF Eu Top Single 23/01/2007 14:57:52 23/01/2007 14:58:01
4 LANCE Eu/APC Top Dual 23/01/2007 14:57:32 23/01/2007 14:57:45
3 FP FITC Dual 23/01/2007 14:56:58 23/01/2007 14:57:08
2 FP FITC Dual 23/01/2007 14:56:46 23/01/2007 14:56:56
1 FI FITC On-the-Fly 23/01/2007 14:56:28 23/01/2007 14:56:33
Press F1 for help
Simulation mode
EnVision

In the view window a number of selection criteria appear. If you have many protocols, use a selection criterion to limit what appears so that you can more easily find the one you want. More than one selection can be used at the same time.

Note! All results of a protocol can be viewed under the protocol in the Result folder.

Results filters

The selections are:

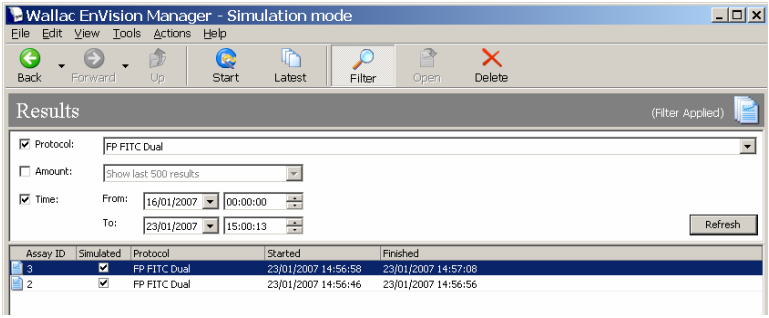
Selection by protocol
Select the protocol from the drop-down list.

Selection by amount
You can select how many of the most recent results are listed.

Selection by date and time
Give the first date and time and the last date and time for the period you want to select.

Note! Each time you make a change to the selection settings, you must click the **Refresh** button. Any assays in the selected range will be listed in a pane below the filter options along with their date and time of running.

The example shows the results of selecting by **Protocol**.



Results toolbar buttons

In the variable part of the ***Results*** toolbar there are three buttons:

Filter
If you click this button once, the filter options disappear leaving only the assay list. Click it again and the filter options reappear.

Open
Clicking this button when you have selected an assay from the list of assays causes the results of the run to be displayed in a separate window. You can also double-click on the result to open it.

Delete
Select the results you want to delete.

You can select several adjacent results by holding down the left mouse button and dragging the cursor over the ones you want. Hold down the Ctrl button and click individual results to select results that are not adjacent. When the results are selected, click the **Delete** button. Confirm the deletion by clicking **OK**.

Note! Results can be restored from the ***Recycle Bin*** provided it has not been emptied

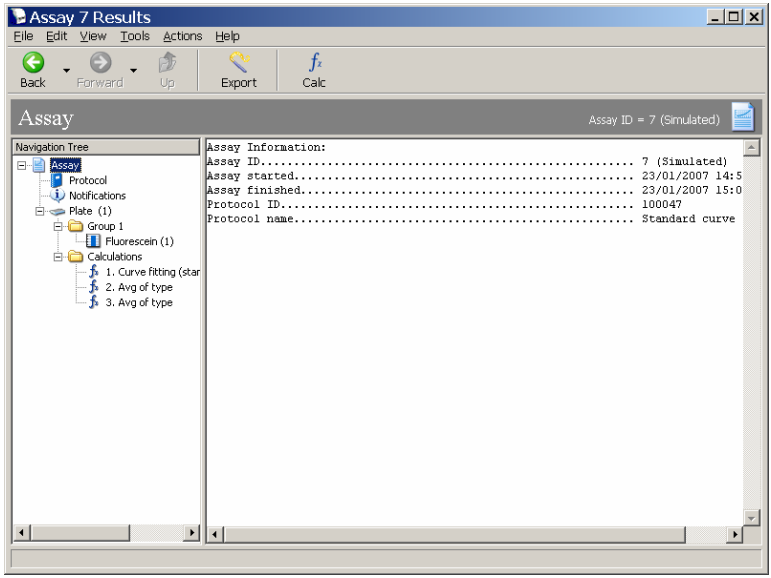
Latest

The **Latest** button enables you to see the results of the most recent run. It opens a separate results window just like the one that appears when you select **Open**.

Results window

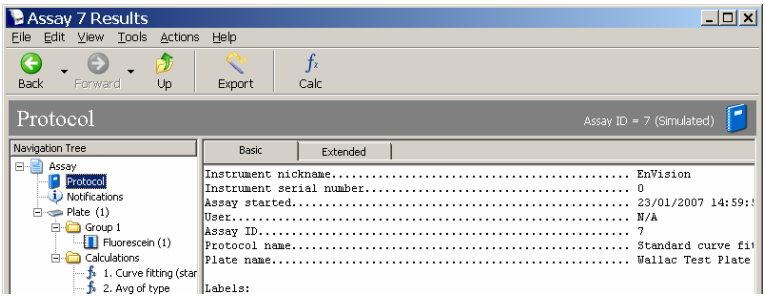
When you open the results window you will see the results tree in the left pane. Click on a branch to see the corresponding view.

Assay

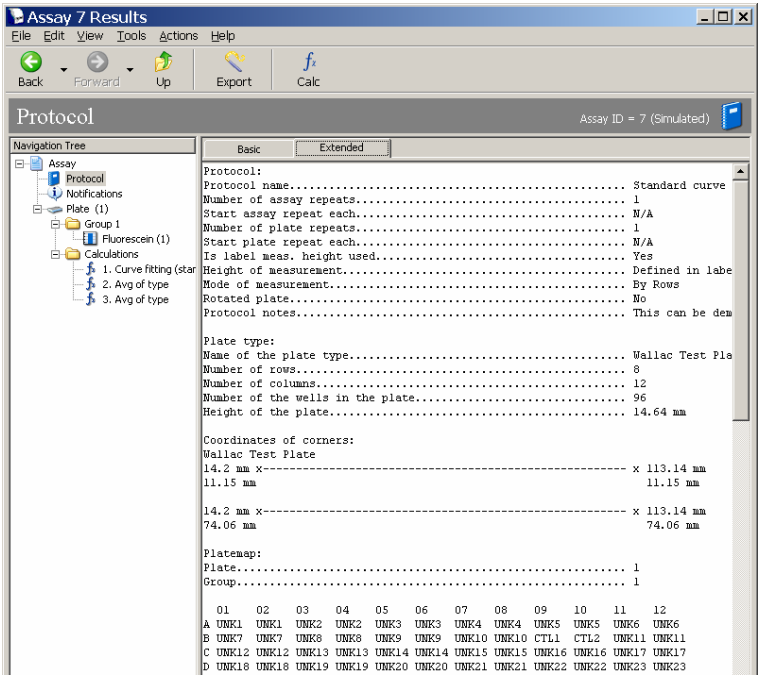


This displays information about the assay.

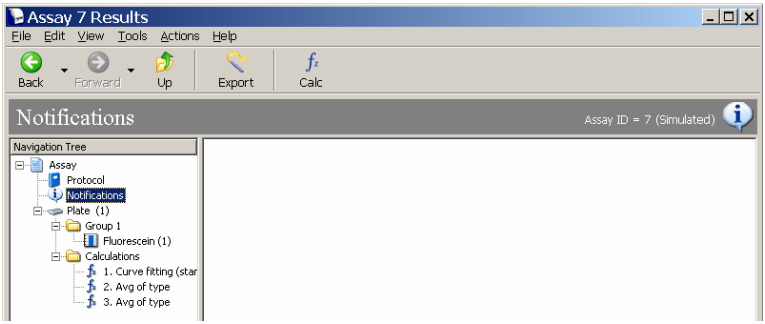
Protocol



This displays the parameters of the protocol used to run the assay. Select either a short list with the **Basic** tab or a longer list with the **Extended** tab.

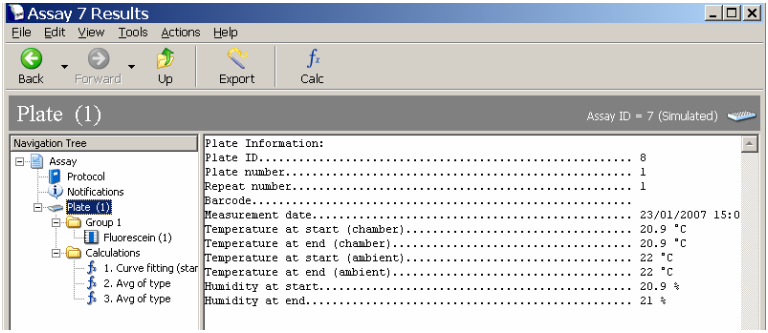

Assay 7 Results
File Edit View Tools Actions Help
Back Forward Up Export Calc
Protocol
Assay ID = 7 (Simulated)
Navigation Tree
Assay
Protocol
Notifications
Plate (1)
Group 1
Fluorescein (1)
Calculations
1. Curve fitting (star
2. Avg of type
3. Avg of type
Basic
Extended
Protocol:
Protocol name..... Standard curve
Number of assay repeats..... 1
Start assay repeat each..... N/A
Number of plate repeats..... 1
Start plate repeat each..... N/A
Is label meas. height used..... Yes
Height of measurement..... Defined in labe
Mode of measurement..... By Rows
Rotated plate..... No
Protocol notes..... This can be dem
Plate type:
Name of the plate type..... Wallac Test Pla
Number of rows..... 8
Number of columns..... 12
Number of the wells in the plate..... 96
Height of the plate..... 14.64 mm
Coordinates of corners:
Wallac Test Plate
14.2 mm x---------- x 113.14 mm
11.15 mm 11.15 mm
14.2 mm x---------- x 113.14 mm
74.06 mm 74.06 mm
Platemap:
Plate..... 1
Group..... 1
01 02 03 04 05 06 07 08 09 10 11 12
A UNK1 UNK1 UNK2 UNK2 UNK3 UNK3 UNK4 UNK4 UNK5 UNK5 UNK6 UNK6
B UNK7 UNK7 UNK8 UNK8 UNK9 UNK9 UNK10 UNK10 CTL1 CTL2 UNK11 UNK11
C UNK12 UNK12 UNK13 UNK13 UNK14 UNK14 UNK15 UNK15 UNK16 UNK16 UNK17 UNK17
D UNK18 UNK18 UNK19 UNK19 UNK20 UNK20 UNK21 UNK21 UNK22 UNK22 UNK23 UNK23

Notifications



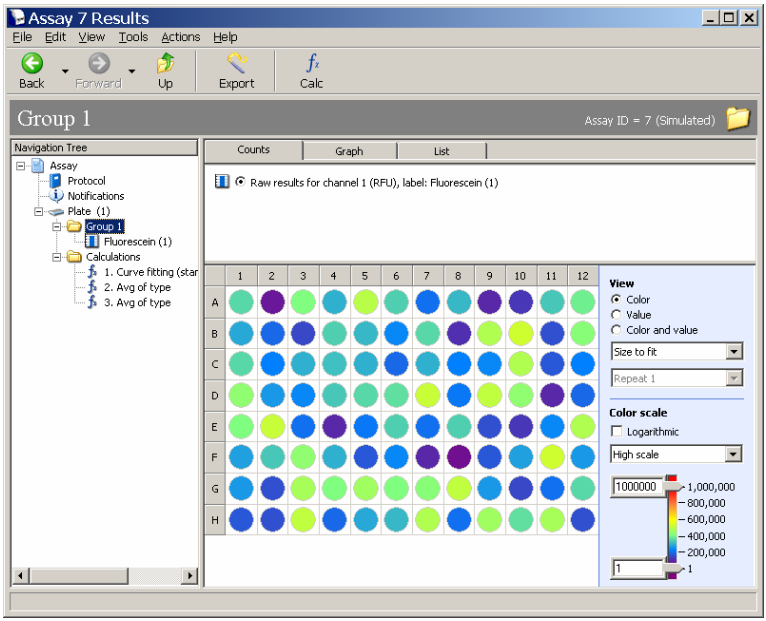
If any error messages or warnings have been produced, they will appear if you click **Notifications**. A yellow exclamation mark shows a warning e.g. if the signal has been too high so that the detector is saturated. A red cross along with the Assay ID icon shows an error. Other notifications are in blue.

Plate

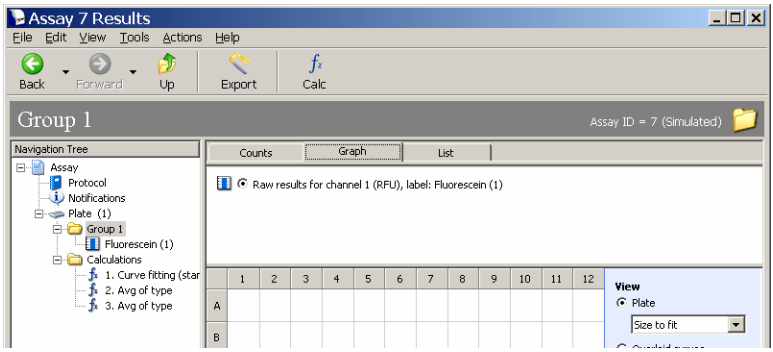


Selecting this will show information about the plate.

Group

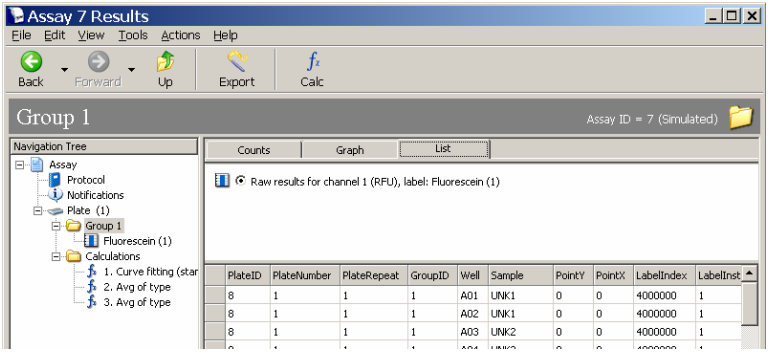


This view has three tabs. The first two, **Counts** and **Graph** (the same as Kinetics) correspond to those described in ***Reader control***.



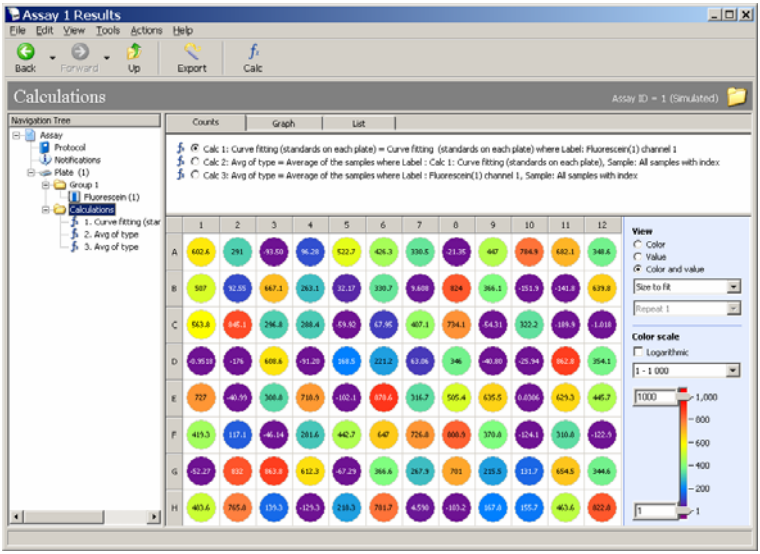
The same features and side-bar functions are available as in ***Reader control***.

The third tab, **List**, shows results in the form of a list of numerical values.


Assay 7 Results
File Edit View Tools Actions Help
Back Forward Up Export Calc
Group 1
Assay ID = 7 (Simulated)
Navigation Tree
Assay
Protocol
Notifications
Plate (1)
Group 1
Fluorescein (1)
Calculations
1. Curve fitting (star
2. Avg of type
3. Avg of type
Counts Graph List
Raw results for channel 1 (RFU), label: Fluorescein (1)
PlateID PlateNumber PlateRepeat GroupID Well Sample PointY PointX LabelIndex LabelInst
8 1 1 1 A01 UNK1 0 0 4000000 1
8 1 1 1 A02 UNK1 0 0 4000000 1
8 1 1 1 A03 UNK2 0 0 4000000 1

Note! In **Group** view, any saturated result will be highlighted with gray under the **Counts** tab.

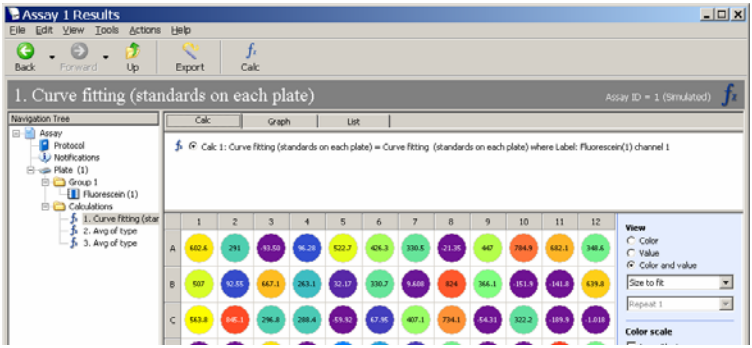
Calculations


Assay 1 Results
File Edit View Tools Actions Help
Back Forward Up Export Calc
Calculations
Assay ID = 1 (Simulated)
Navigation Tree
Assay
Protocol
Notifications
Plate (1)
Group 1
Fluorescein (1)
Calculations
1. Curve fitting (star
2. Avg of type
3. Avg of type
Counts
Graph
List
Calc 1: Curve fitting (standards on each plate) = Curve fitting (standards on each plate) where Label: Fluorescein(1) channel 1
Calc 2: Avg of type = Average of the samples where Label : Calc 1: Curve fitting (standards on each plate), Sample: All samples with index
Calc 3: Avg of type = Average of the samples where Label : Fluorescein(1) channel 1, Sample: All samples with index
1 2 3 4 5 6 7 8 9 10 11 12
A 602.6 291 93.50 96.28 522.7 426.3 330.5 -21.35 447 784.9 682.1 348.6
B 507 92.05 647.1 263.1 32.17 330.7 9.608 824 366.1 -151.9 -141.8 639.8
C 563.8 645.1 296.8 288.4 69.92 67.95 407.1 754.1 64.31 322.2 -189.9 -1.018
D -0.9518 -176 608.6 -91.20 268.5 221.2 63.06 346 -40.80 -25.94 862.8 354.1
E 727 -40.59 366.8 716.9 -102.1 879.6 316.7 505.4 635.5 0.6366 629.3 445.7
F 419.3 117.1 -46.14 281.6 442.7 647 726.8 600.5 370.8 -124.1 310.8 -122.9
G -52.27 832 963.8 612.3 -67.29 366.6 267.9 701 235.5 131.7 654.5 344.6
H 403.6 765.8 155.3 -129.3 218.3 781.7 4.536 -103.2 167.6 155.7 463.6 822.8
View
Color
Value
Color and value
Size to fit
Repeat 1
Color scale
Logarithmic
1 - 1 000
1000
1,000
800
600
400
200
1

All the calculations selected for the protocol are listed.

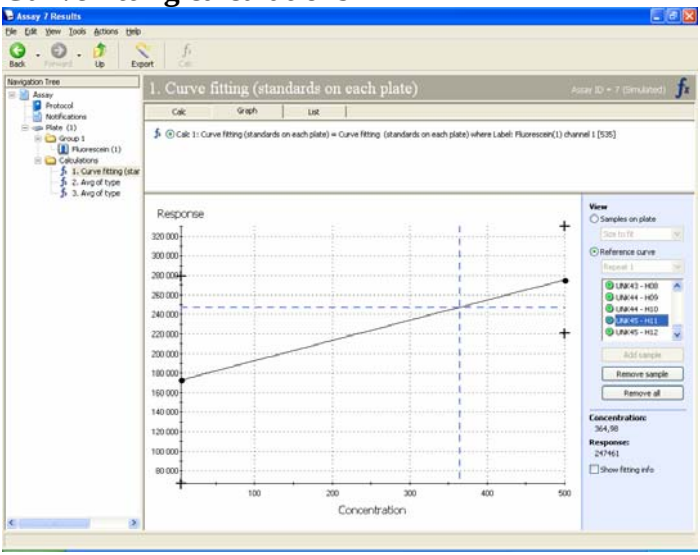
Note! If a calculation error has appeared in **Notifications**, the calculation that has been defined might disappear from the list with no results shown.

To see the results of these calculations, click on them one by one in the navigation tree. Each time you select a calculation, the main pane in the window will show the results of the calculation.


Assay 1 Results
File Edit View Tools Actions Help
Back Forward Up Export Calc
1. Curve fitting (standards on each plate)
Navigation Tree
Calc Graph List
Calc 1: Curve fitting (standards on each plate) = Curve fitting (standards on each plate) where Label: Fluorescein(1) channel 1
View
Color
Value
Color and value
Size to fit
Color scale

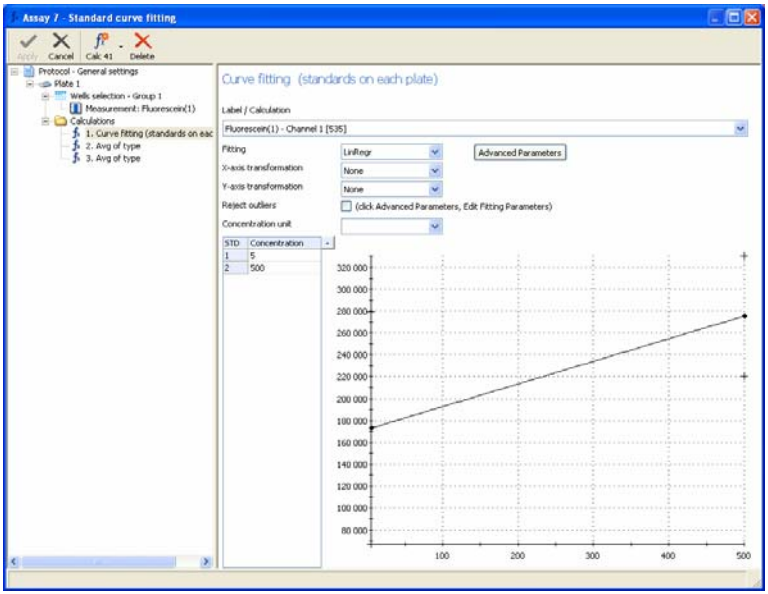
Normally the **Counts** view will be open but if the calculation is curve fitting, the **Graph** view will open automatically to show the curve. This shows the points from the standards that were measured and the curve fitted to them. Clicking the **Show fitting info** check box causes additional information to appear beside the curve.

Curve fitting calculations

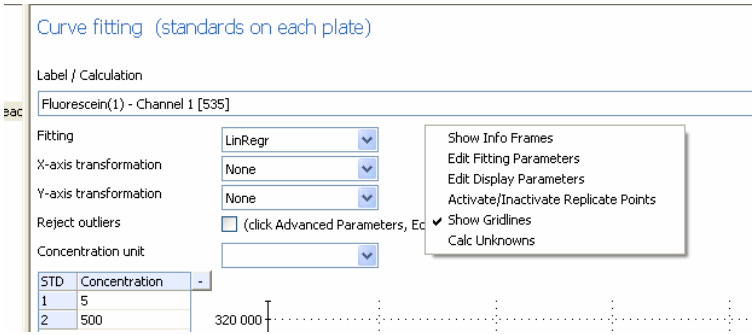


If you click on one of the unknowns listed in the right hand pane, the intercept with the curve from the response of the unknown and the appropriate concentration will be shown on the graph. If the response of the unknown is outside that of the range of the calibration curve, no intercepts will be shown on the graph. The response and concentration values will be listed in red instead of the normal black.

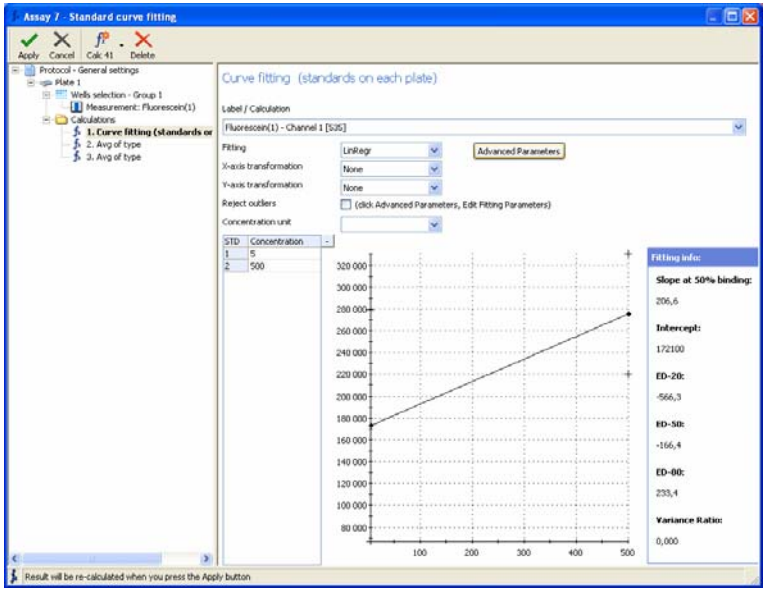
To edit the curve, select the **Calc** button from the toolbar. A new window appears showing a curve and various curve fitting parameters (see **Advanced parameters**) as well as the concentrations of the standards and the units.



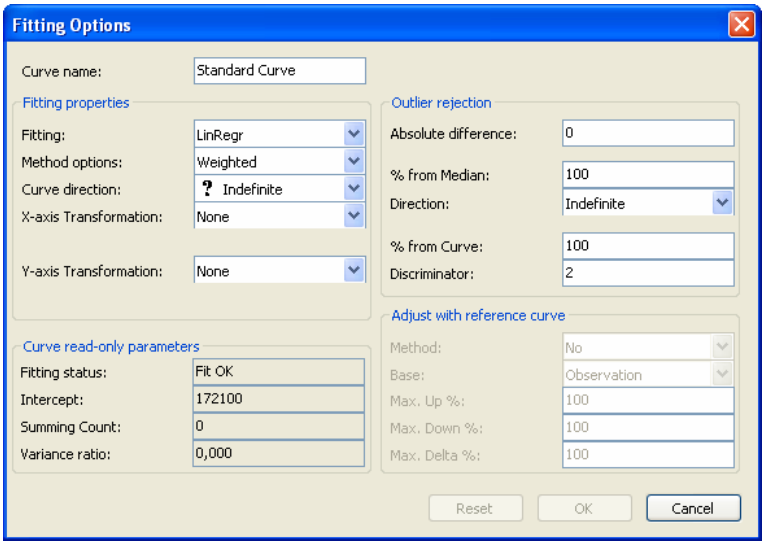
There is a button **Advanced parameters** giving you the following options:



Show info frames – an extra pane appears with more information about the curve including the slope, the intercept, the estimated dose at various percentages and the variance.



Edit fitting parameters – a dialog appears allowing you to set many parameters which affect the curve fitting. Some of the parameters to do with curve fitting also appear in the main calculation window.



Fitting: select from the following types:
Linear regression
Spline – in this case the Spline smooth parameter is enabled
4PL
5PL
MichaelisMenten

Spline smoothing: this only appears if Spline is selected. You can select the amount of smoothing applied to the Spline curve from simple interpolation

through various percentages of smoothing to full auto-smoothing

Weighted fit: click this check box if you want the fit to be weighted

Curve direction: select if the curve must go up or down or without a special direction

Transformations for the X and Y axes: Hyperbolic, Logarithmic or None in the case of the X axis and many more in the case of the Y axis

Scale factor for the X and Y axes: for hyperbolic transformations select from: Log, 0.1,1 or 10

Curve read only parameters: these cannot be changed, they just tell about the: Fitting status, Intercept, Summing count and Variance ratio

Curve name: You can edit the name in this field if you want

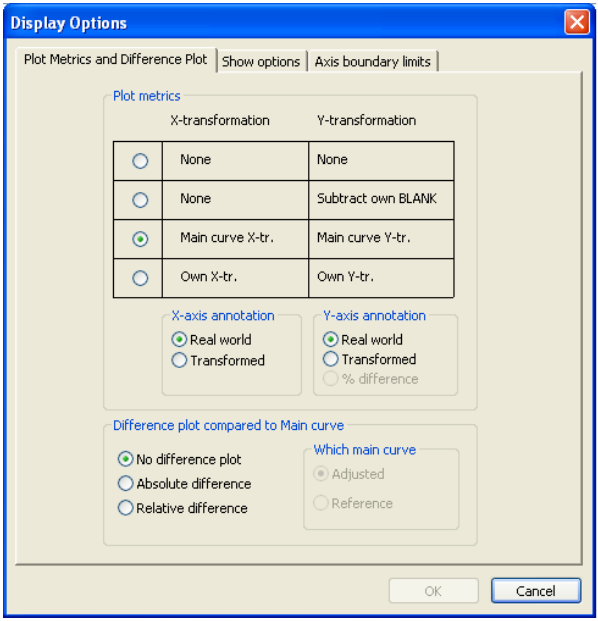
Outlier rejection: you can set criteria for rejecting outliers. If a point satisfies one of these criteria then it will be rejected and not included in the curve

Adjust with reference curve: the default selection is **No** but you can also select **Constant** or **Linear**. In these cases the other parameters are enabled so that you can edit them if required.

Edit display parameters: you can adjust how the curve is displayed by changing these parameters. You can

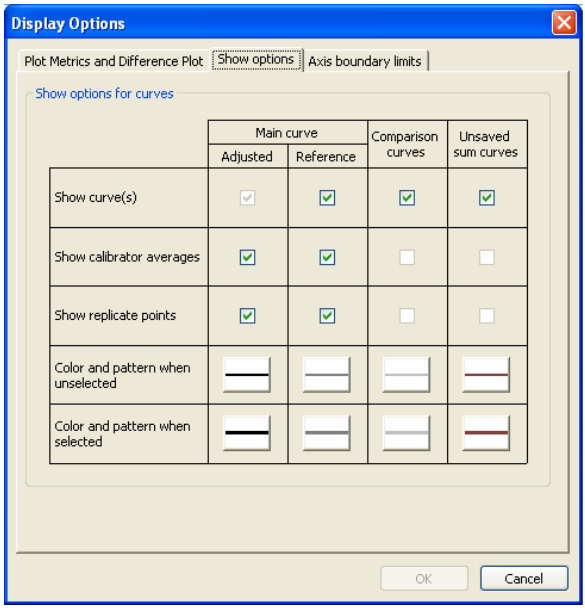
experiment with the options and check boxes to see which one gives you the display you want.

There are three tabs.

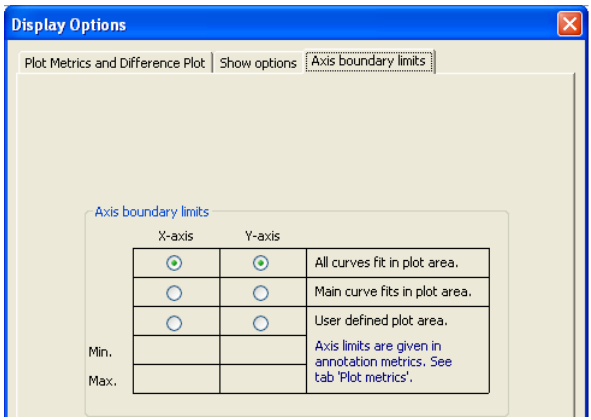


In the first one the option buttons allow you to select which metrics are to be used and if a difference plot is displayed.

The next tab shows check boxes which determine the way the curve is displayed.

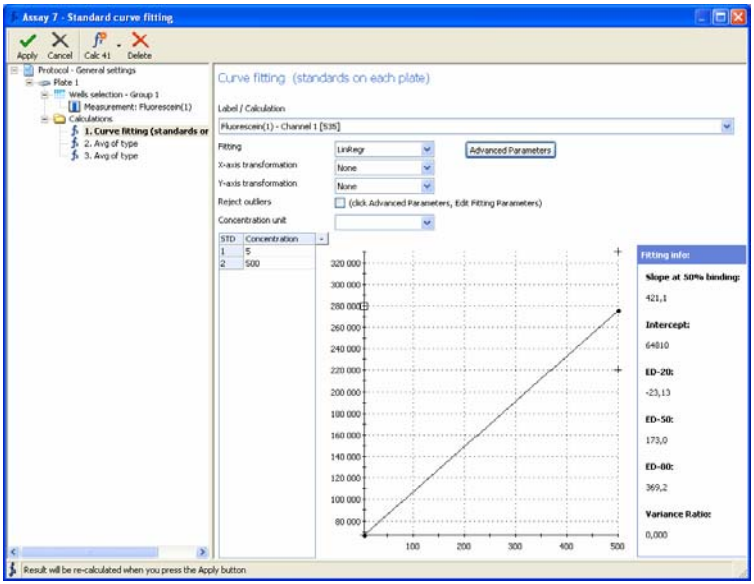


The last tab gives you options to select the axis boundary limits.

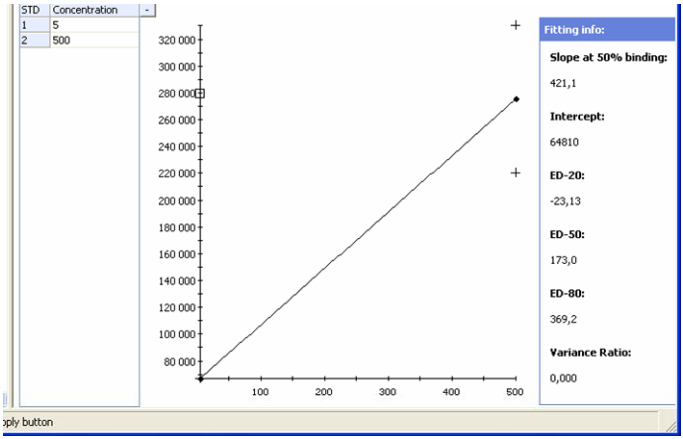


Activate/Inactivate replicate points: if you want to see the effect of omitting a replicate point, you can do this by unchecking the box for the point.

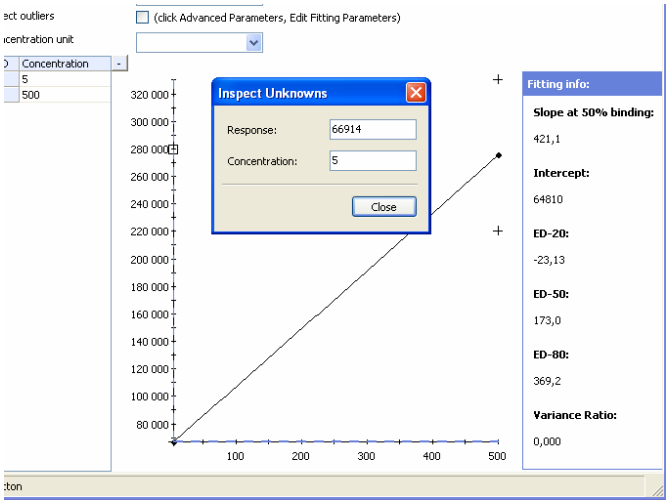
The unused point appears as an open square on the graph instead of a cross.



Show gridlines: this toggles the gridlines on and off

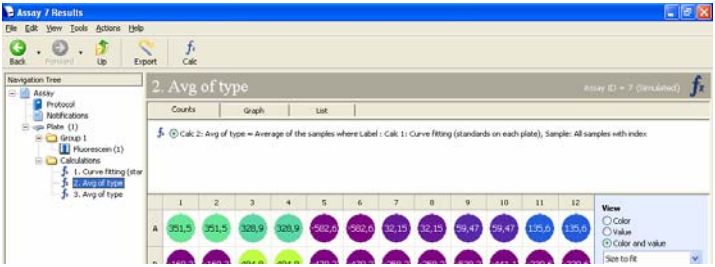


Calc unknowns: type in a response value and see the concentration calculated from the curve. The response value must be within the range of the curve

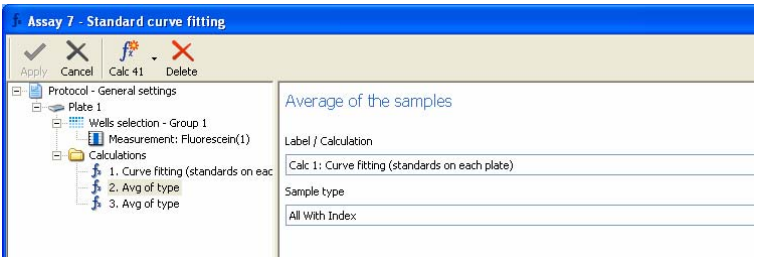


Other types of calculations

If you select a calculation that does not use a curve e.g. Average of type, then the display is different:

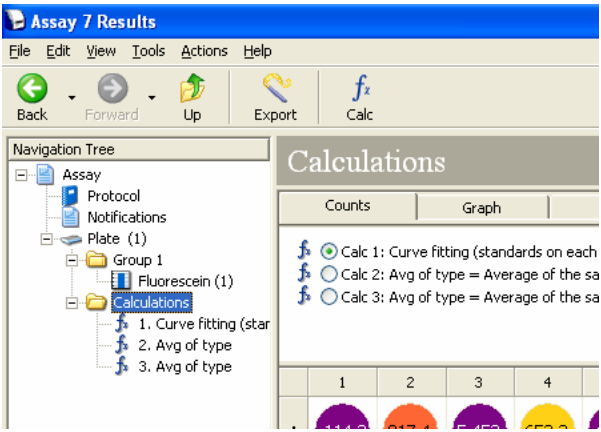

Assay 7 Results
File Edit View Tools Actions Help
Back Forward Up Export Calc
Navigation Tree
Assay
Protocol
Notifications
Plate (1)
Group 1
Fluorescein (1)
Calculations
1. Curve fitting (star
2. Avg of type
3. Avg of type
2. Avg of type
Counts Graph List
Calc 2: Avg of type = Average of the samples where Label : Calc 1: Curve fitting (standards on each plate), Sample: All samples with index
1 2 3 4 5 6 7 8 9 10 11 12
A 351,5 351,5 328,9 328,9 562,6 562,6 32,15 32,15 59,47 59,47 135,6 135,6
View
Color
Value
Color and value
Size to fit

If you now click the **Calc** button, the display shows details of the calculation:

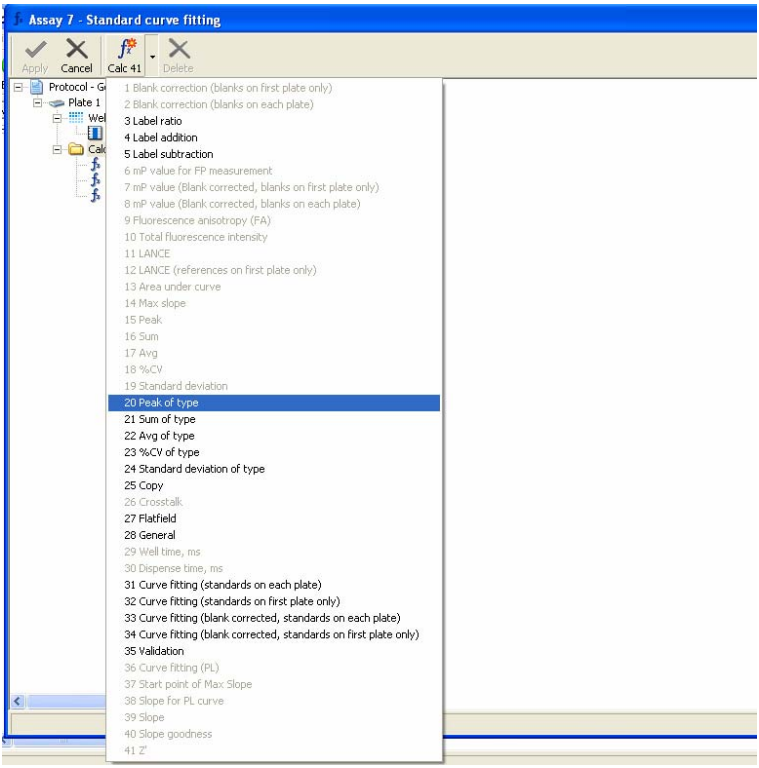


Adding additional calculations

Note! If a calculation was not added to the protocol prior to measurement, it can be done afterward. First click the **Calc** button in the ***Results*** window.

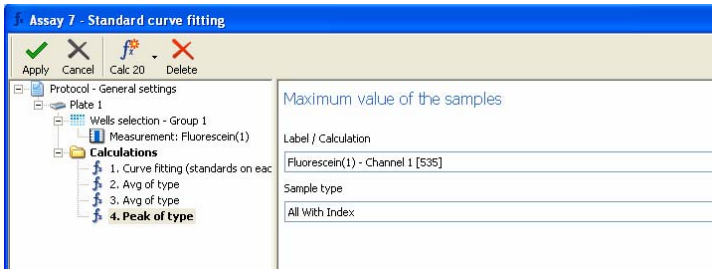


In the calculation window that appears, select the arrow beside the **Calc** button to see the list of calculations:


Assay 7 - Standard curve fitting
Apply
Cancel
Calc 41
Delete
Protocol - G
Plate 1
1 Blank correction (blanks on first plate only)
2 Blank correction (blanks on each plate)
3 Label ratio
4 Label addition
5 Label subtraction
6 mP value for FP measurement
7 mP value (Blank corrected, blanks on first plate only)
8 mP value (Blank corrected, blanks on each plate)
9 Fluorescence anisotropy (FA)
10 Total fluorescence intensity
11 LANCE
12 LANCE (references on first plate only)
13 Area under curve
14 Max slope
15 Peak
16 Sum
17 Avg
18 %CV
19 Standard deviation
20 Peak of type
21 Sum of type
22 Avg of type
23 %CV of type
24 Standard deviation of type
25 Copy
26 Crosstalk
27 Flatfield
28 General
29 Well time, ms
30 Dispense time, ms
31 Curve fitting (standards on each plate)
32 Curve fitting (standards on first plate only)
33 Curve fitting (blank corrected, standards on each plate)
34 Curve fitting (blank corrected, standards on first plate only)
35 Validation
36 Curve fitting (PL)
37 Start point of Max Slope
38 Slope for PL curve
39 Slope
40 Slope goodness
41 Z'

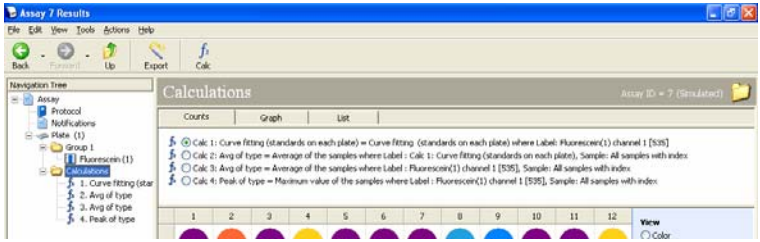
Note! The calculations available depend on the measured labels and the measurement operations.

Select the calculation type you want to add. The new calculation will be added to the calculation list.



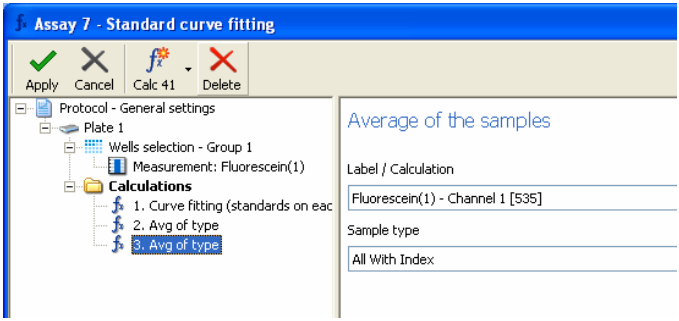
There may be parameters you need to set. See "Calculations" in the Reference manual for more information.

Click **Apply** to obtain results using the calculation. The ***Results*** window will open with the additional calculation added.



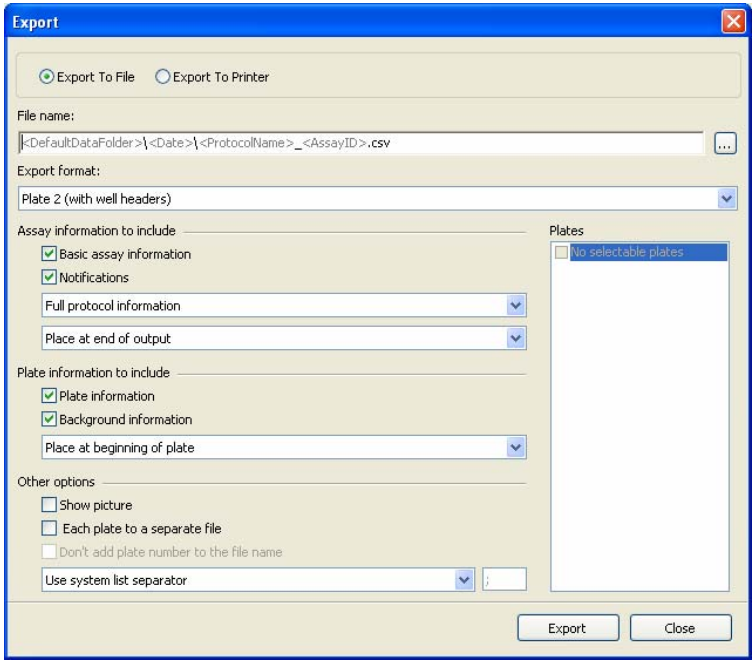
Note! The raw data is not changed by adding or removing calculations.

To delete a calculation, click the **Calc** button to get the calculation window. Select the calculation you want to delete, then click the **Delete** button then click **Apply**.



If you click the **Cancel** button instead of **Apply**, the calculation window will close without making changes.

Export



Clicking this button on the toolbar allows you to send results to a file and/or printer. The options for the format of the output are the same as described in ***Protocol* Output**.

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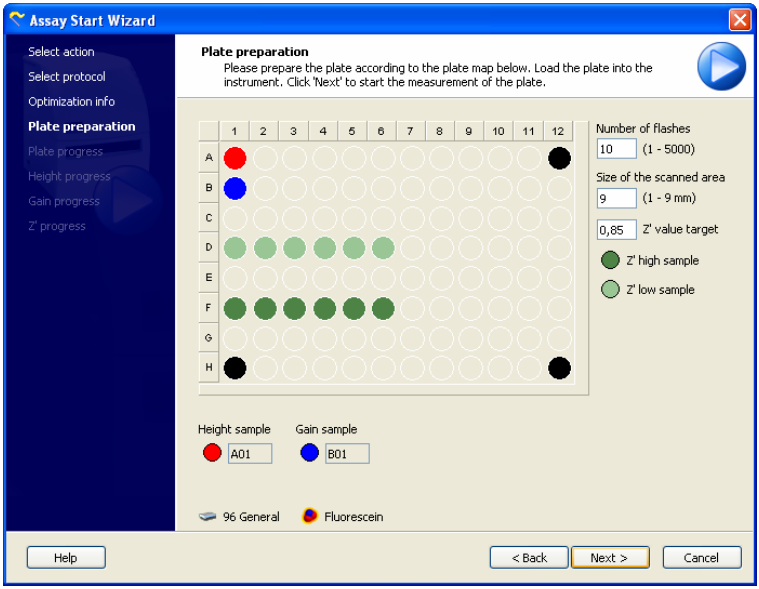
Chapter 4
Advanced Optimization

Advanced Optimization

These windows normally appear only if you selected **Advanced** in the **Optimization Info** window of the **Assay Start Wizard**. Only the additional advanced windows are described here, otherwise operation is as described in **Assay Start Wizard**. In normal optimization you would not change these parameters but you may want to as part of **Advanced** optimization.

Plate dimension optimization



The plate map parameters are as follows.

Number of flashes
You can select the number of flashes to be used. This can be different from the number set in the label parameters. E.g. if in the label parameters the number of flashes is set to 30 you can change it to e.g. 10 to speed up the optimization process. With AlphaScreen the measurement time is fixed and it cannot be changed. The measurement is made with an AlphaScreen sample.

Size of scanned area
Enter the size of the edges of the area of the wells to be scanned. See the table for typical size settings.

Size settings

No. of wells	**Spacing of centers (mm)**	**Typical size setting (mm)**	**Allowed range (mm)**
1536	2.25	1.8	1 - 4.5
384	4.5	2.7	1 - 9.0
96	9	6.3	1 - 18

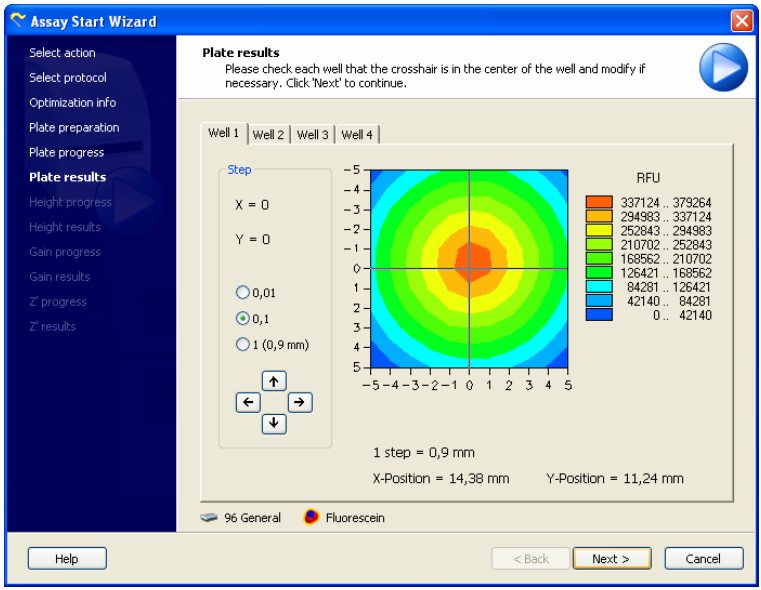
Note! There are 10 movements in the scan (in both the horizontal and vertical directions producing a measurement of 121 points in an 11 x 11 array).

Z’ value target
Give the Z’ target value. This is typically between 0.6 and 1.0.

When you click **Next** the optimization will begin.

If Plate optimization has been selected a window will appear with four tabs each with a picture showing the distribution of signal intensity across a corner well. These pictures are to enable you to mark the center of each well. This will be the area with the most intense signal (in the case of absorbance the signal will be lowest here). Color coded contours show the signal intensity with red the most intense.

The order of the wells is:
Well 1 - upper left corner
Well 2 - upper right corner
Well 3 - lower left corner
Well 4 - lower right corner



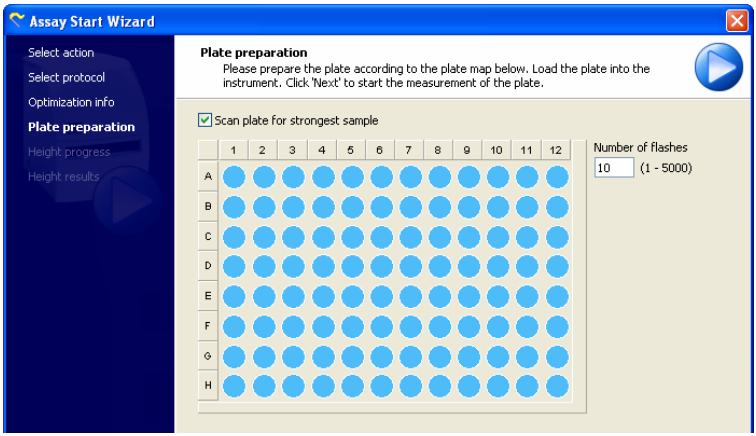
Note! In fluorescence intensity and fluorescence polarization measurements, the signal should not exceed 500 000 RFUs for a single flash, otherwise saturation of the detector may occur and you will not be able to be sure of the position of greatest intensity. In this case, and if the red area is not symmetrical, you should point to the center of the corner well as indicated by the general shape of the contours.

The crosshairs should be in the centre of the well. If they are not, you can move them with the mouse or by clicking the **Step** arrows. You can also select the size of the step.

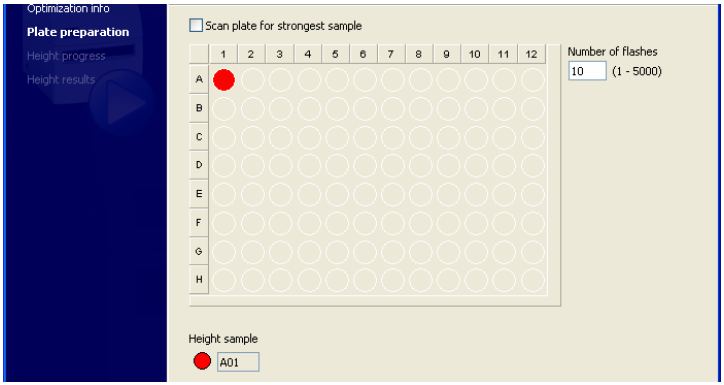
When you are satisfied with the position for each well, click **Next**.

Measurement height optimization

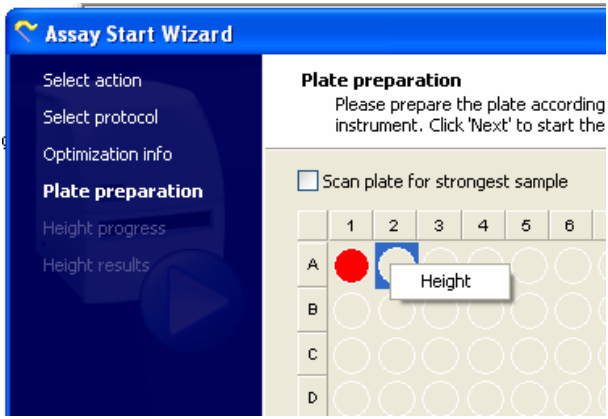
If you leave the **Scan plate for strongest sample** check box checked then the position of the sample to be used for the measurement height optimization is determined by the system.


Assay Start Wizard
Select action
Select protocol
Optimization info
Plate preparation
Height progress
Height results
Plate preparation
Please prepare the plate according to the plate map below. Load the plate into the instrument. Click 'Next' to start the measurement of the plate.
Scan plate for strongest sample
1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H
Number of flashes
10
(1 - 5000)

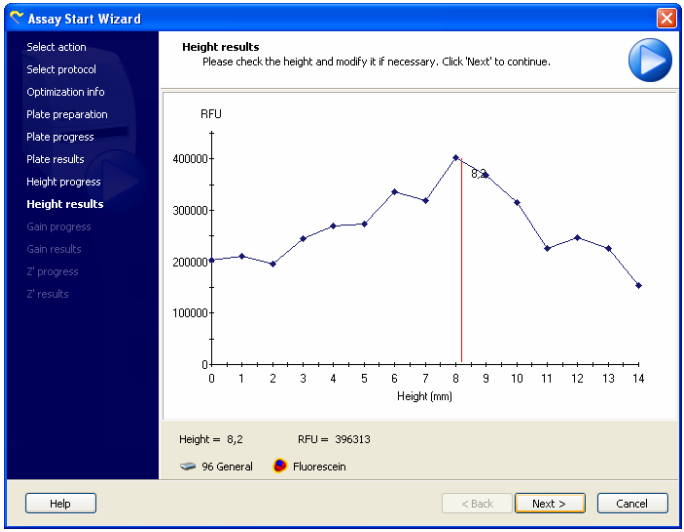
If you uncheck this box, you can either accept the default position which is shown in the plate map



or you can show the position of the sample you want to use by clicking on the plate map using the left mouse button and selecting the word Height that appears in the tool tip.



When you click **Next**, the optimization will begin.



An additional window will appear to allow you to manually adjust the optimum height of the plate. Click the height you want to select. The red line will move to that position and the height at that position will be displayed.

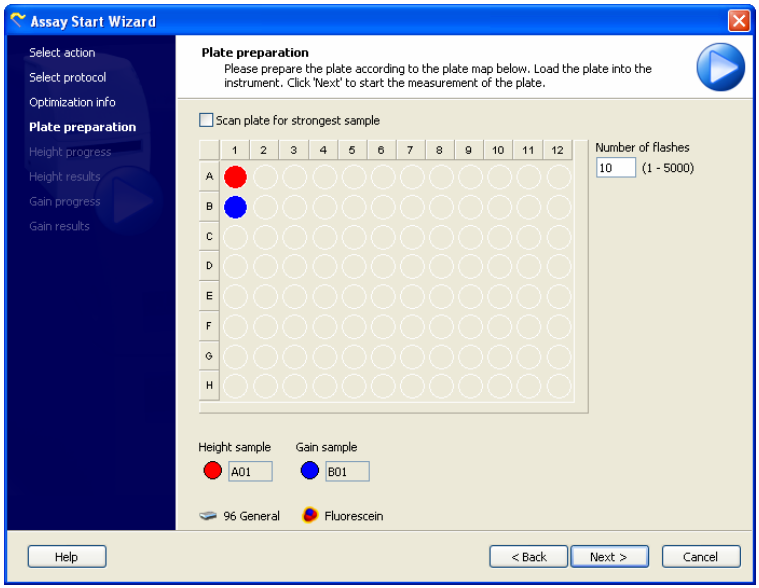
Click **Next** to continue.

Detector gain optimization (FI and FP)

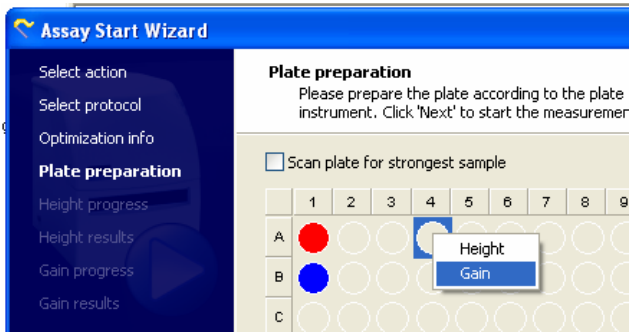
If you leave **Scan plate for strongest sample** checked, the position of the gain sample is determined by the system.

Note! When you use the **Scan plate for strongest sample** option in fluorescence polarization, it is recommended that you check with a low binding assay sample that the S and P signal levels are in a suitable range (200 000 - 400 000 per flash) and that they match the target mP value which is shown as part of the optimization results.

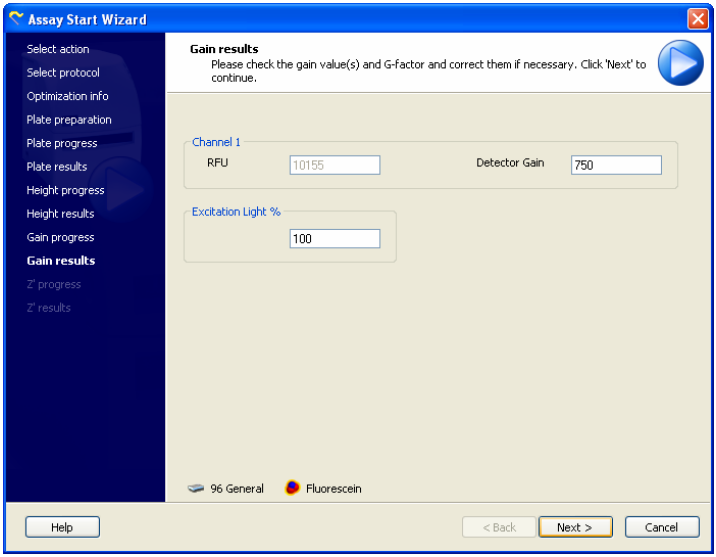
If you uncheck this box, you can either accept the default position that is shown in the plate map (along with the Height sample if that optimization has been accepted)



or you can show the position(s) of the sample(s) you want to use by clicking on the plate map using the left mouse button. Select the sample type, **Height** or **Gain**.



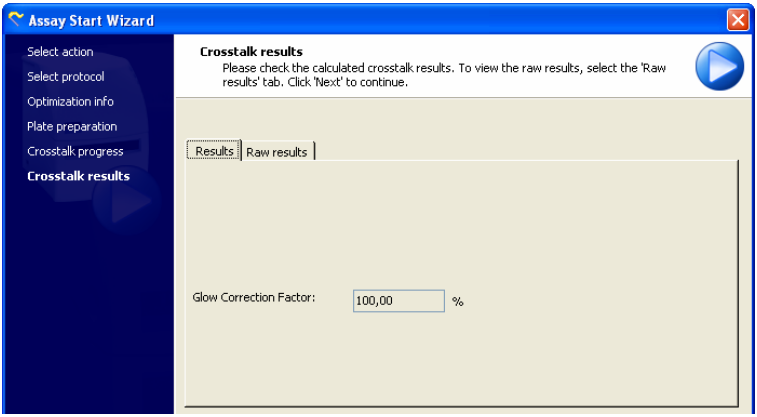
When you click **Next**, the optimization will begin. An additional window will appear to allow you to check and, if you want, to adjust the gain.



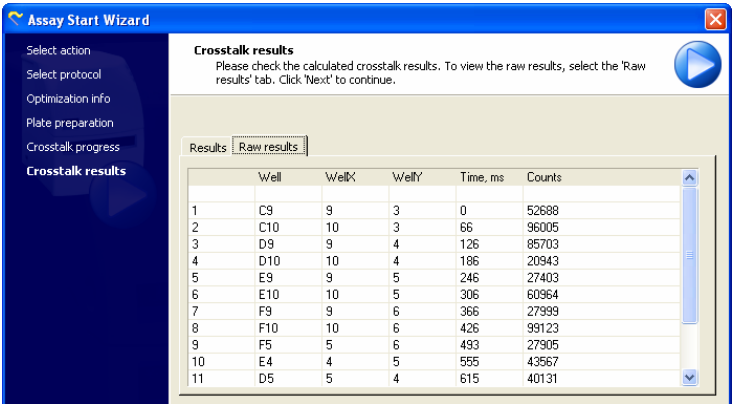
Click **Next** to go to the final window of the wizard.

Crosstalk correction optimization (Enh. lum. and Ultra Sensitive lum.)

After you have run the optimization, as described in **Assay Start Wizard**, the results will appear:



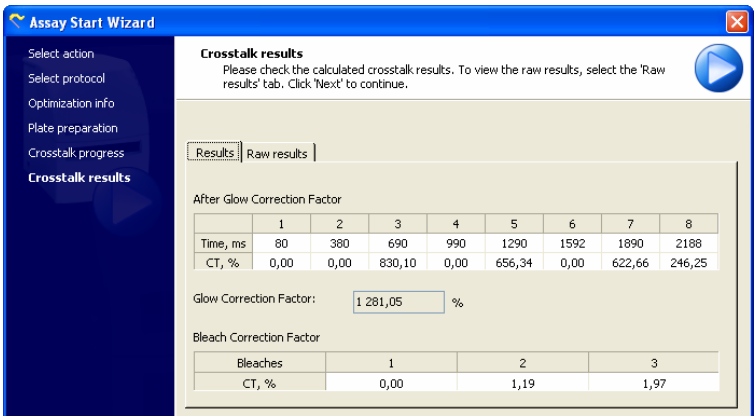
You can also click the tab to see the **Raw** results.


Assay Start Wizard
Select action
Select protocol
Optimization info
Plate preparation
Crosstalk progress
Crosstalk results
Crosstalk results
Please check the calculated crosstalk results. To view the raw results, select the 'Raw results' tab. Click 'Next' to continue.
Results
Raw results
Well
WellX
WellY
Time, ms
Counts
1 C9 9 3 0 52688
2 C10 10 3 66 96005
3 D9 9 4 126 85703
4 D10 10 4 186 20943
5 E9 9 5 246 27403
6 E10 10 5 306 60964
7 F9 9 6 366 27999
8 F10 10 6 426 99123
9 F5 5 6 493 27905
10 E4 4 5 555 43567
11 D5 5 4 615 40131

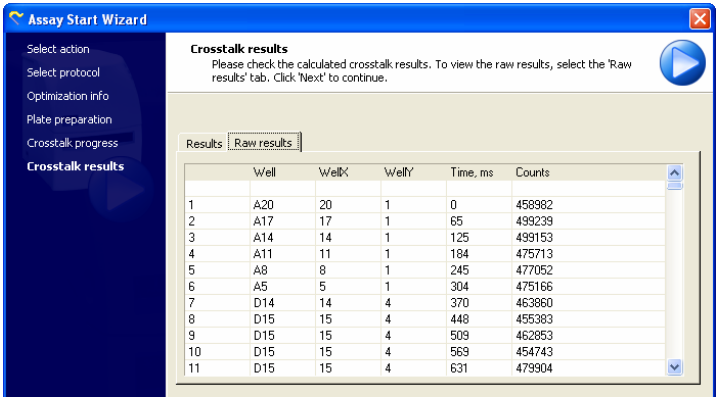
Click **Next** to go to the final window of the wizard.

Crosstalk correction optimization (AlphaScreen and HTS AlphaScreen)

After you have run the optimization, as described in **Assay Start Wizard**, the results will appear:


Assay Start Wizard
Select action
Select protocol
Optimization info
Plate preparation
Crosstalk progress
Crosstalk results
Crosstalk results
Please check the calculated crosstalk results. To view the raw results, select the 'Raw results' tab. Click 'Next' to continue.
Results
Raw results
After Glow Correction Factor
1 2 3 4 5 6 7 8
Time, ms 80 380 690 990 1290 1592 1890 2188
CT, % 0,00 0,00 830,10 0,00 656,34 0,00 622,66 246,25
Glow Correction Factor: 1 281,05 %
Bleach Correction Factor
Bleaches 1 2 3
CT, % 0,00 1,19 1,97

You can also click the tab to see the **Raw** results.


Assay Start Wizard
Select action
Select protocol
Optimization info
Plate preparation
Crosstalk progress
Crosstalk results
Crosstalk results
Please check the calculated crosstalk results. To view the raw results, select the 'Raw results' tab. Click 'Next' to continue.
Results
Raw results
Well WellX WellY Time, ms Counts
1 A20 20 1 0 458982
2 A17 17 1 65 499239
3 A14 14 1 125 499153
4 A11 11 1 184 475713
5 A8 8 1 245 477052
6 A5 5 1 304 475166
7 D14 14 4 370 463860
8 D15 15 4 448 455383
9 D15 15 4 509 462853
10 D15 15 4 569 454743
11 D15 15 4 631 479904

Click **Next** to go to the final window of the wizard.

Chapter 5.
Dispenser control

Dispenser Control

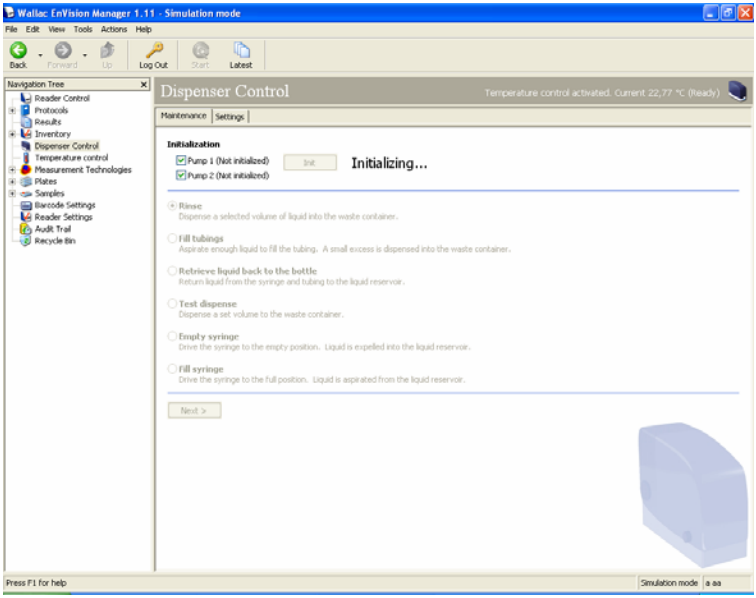
Click ***Dispenser Control*** to access functions and parameters involved in the operation of the dispenser. There are two tabs: Maintenance and Settings.

Maintenance

You can perform maintenance operations with the dispenser by selecting the pump and the operation.

Initialization

Select the pump or pumps to be initialized then click the **Init** button to perform initialization.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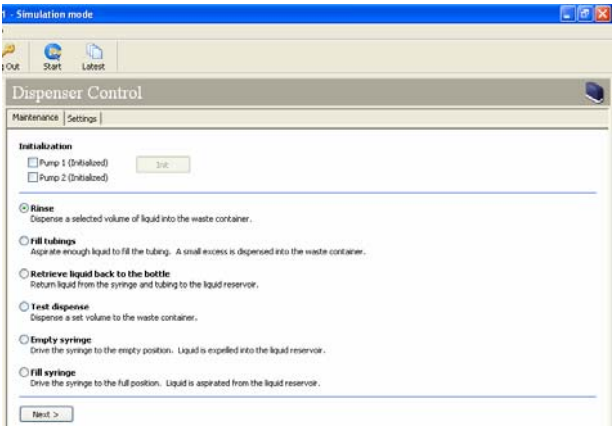
Dispenser Control
Temperature control activated. Current 22,77 °C (Ready)
Maintenance Settings
Initialization
Pump 1 (Not initialized)
Pump 2 (Not initialized)
Init
Initializing...
Rinse
Dispense a selected volume of liquid into the waste container.
Fill tubings
Aspirate enough liquid to fill the tubing. A small excess is dispensed into the waste container.
Retrieve liquid back to the bottle
Return liquid from the syringe and tubing to the liquid reservoir.
Test dispense
Dispense a set volume to the waste container.
Empty syringe
Drive the syringe to the empty position. Liquid is expelled into the liquid reservoir.
Fill syringe
Drive the syringe to the full position. Liquid is aspirated from the liquid reservoir.
Next >
Press F1 for help
Simulation mode
a aa

Initialization resets the pumps by setting the valves and syringes to their home positions. This may involve liquid being expelled through the aspiration tube into the liquid reservoir. If you are not sure what liquid was used last, direct the aspiration tube into an empty reservoir to avoid accidentally mixing liquids.

Note!: the other maintenance operations are disabled until **Init** has been clicked and initialization performed.

Rinse


- Simulation mode
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Initialization
Pump 1 (Initialized)
Pump 2 (Initialized)
Init
Rinse
Dispense a selected volume of liquid into the waste container.
Fill tubings
Aspirate enough liquid to fill the tubing. A small excess is dispensed into the waste container.
Retrieve liquid back to the bottle
Return liquid from the syringe and tubing to the liquid reservoir.
Test dispense
Dispense a set volume to the waste container.
Empty syringe
Drive the syringe to the empty position. Liquid is expelled into the liquid reservoir.
Fill syringe
Drive the syringe to the full position. Liquid is aspirated from the liquid reservoir.
Next >

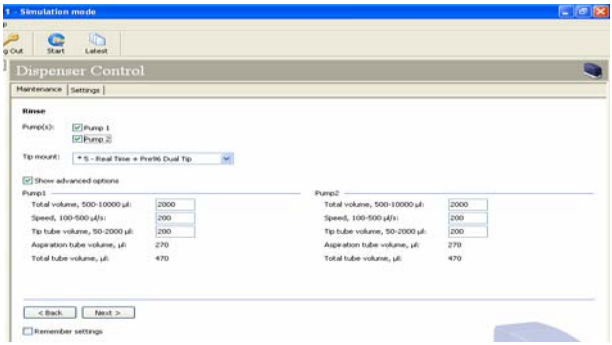
This operation allows you to dispense a selected volume of liquid into the waste container. You can use this operation to empty tubing if you perform it with the inlet tube in air.

Click **Next** to continue.

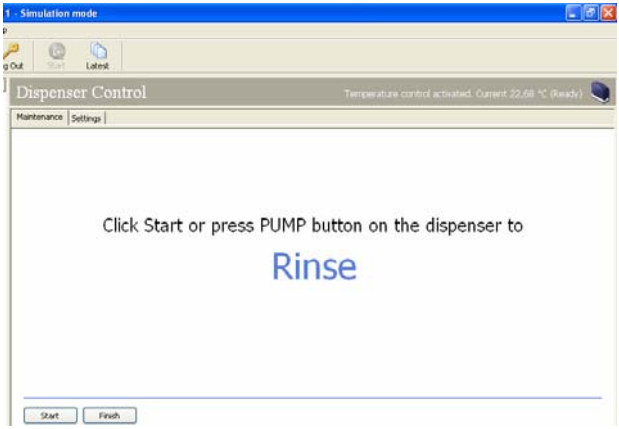
This operation allows you to rinse the tubing of the selected pump or pumps.

Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

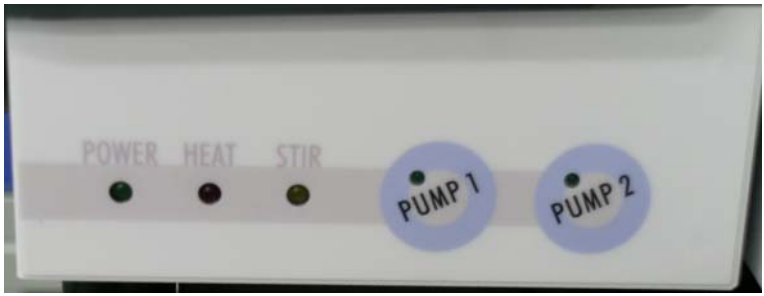
See **Show advanced options** for some extra parameters you can edit.



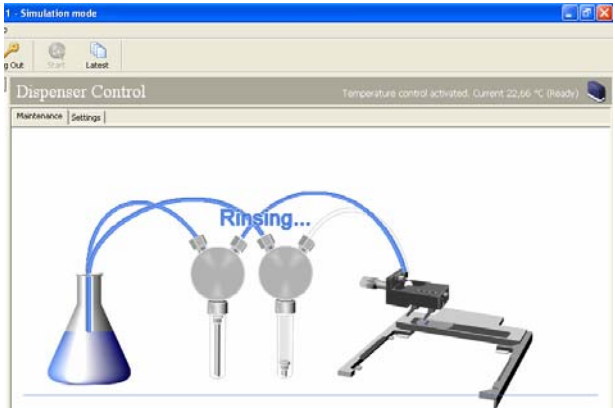
Click **Next** to continue or **Back** to return to the **Maintenance** page.



Click **Start** or press the PUMP1 button on the dispenser. Rinsing will then take place.



An animation will show what is happening.



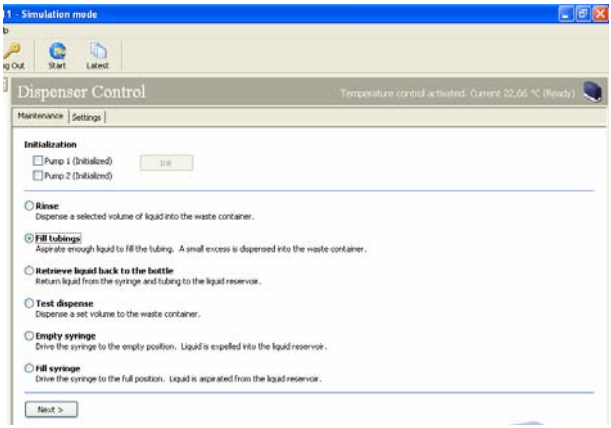
You can repeat this operation if required to rinse with PUMP2.

Click **Finish**. The **Maintenance** display will reappear.

Note! If any PUMP button is pressed while the dispenser is operating, that operation will be terminated immediately and dispenser initialization must be done again.

Fill tubing

This operation aspirates enough liquid to fill the tubing. A small extra amount (about 150 µL) will be aspirated to ensure there are no air bubbles in the tubing. The excess will be dispensed into the waste container.


Simulation mode
Start
Latest
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Temperature control activated. Current 22,66 °C (Ready)
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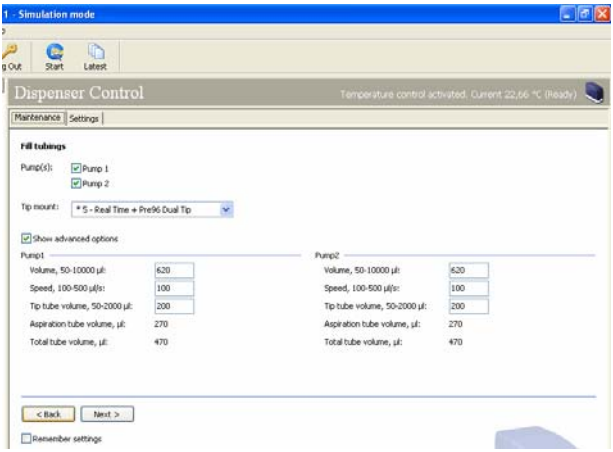
Settings
Initialization
Pump 1 (Initialized)
Pump 2 (Initialized)
Rinse
Dispense a selected volume of liquid into the waste container.
Fill tubings
Aspirate enough liquid to fill the tubing. A small excess is dispensed into the waste container.
Retrieve liquid back to the bottle
Return liquid from the syringe and tubing to the liquid reservoir.
Test dispense
Dispense a set volume to the waste container.
Empty syringe
Drive the syringe to the empty position. Liquid is expelled into the liquid reservoir.
Fill syringe
Drive the syringe to the full position. Liquid is aspirated from the liquid reservoir.
Next >

Click **Next** when you have selected this option.

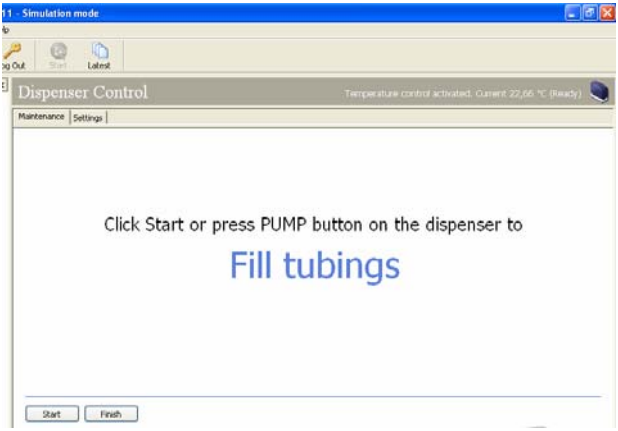
This operation allows you to fill the tubing of the selected pump or pumps.

Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

See **Show advanced options** for some extra parameters you can edit.

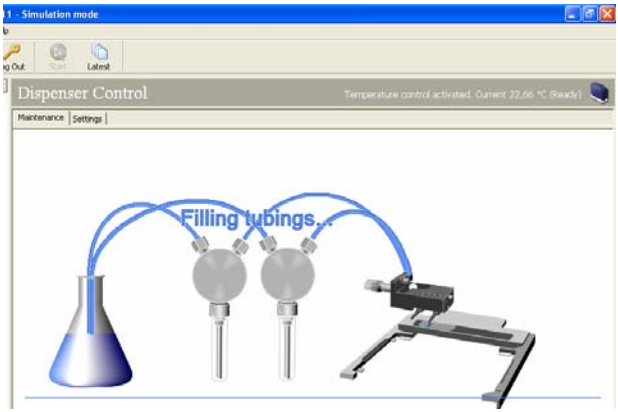


Click **Next**.



Click **Start** or press the appropriate PUMP button on the dispenser. Tubing will be filled.

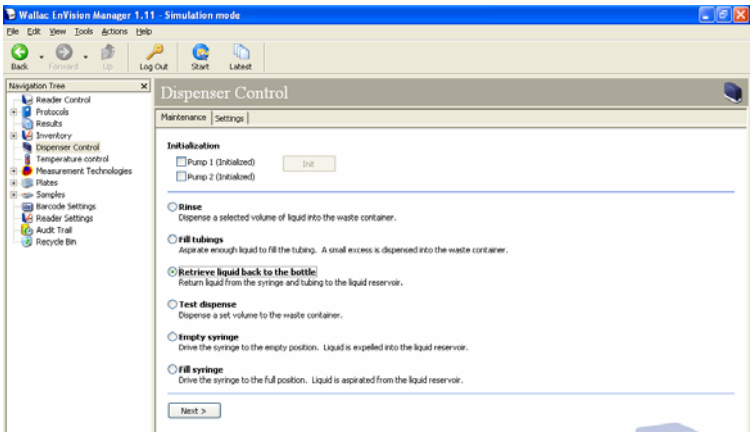
An animation will show what is happening.



You can repeat this operation if required to fill tubing for PUMP2.

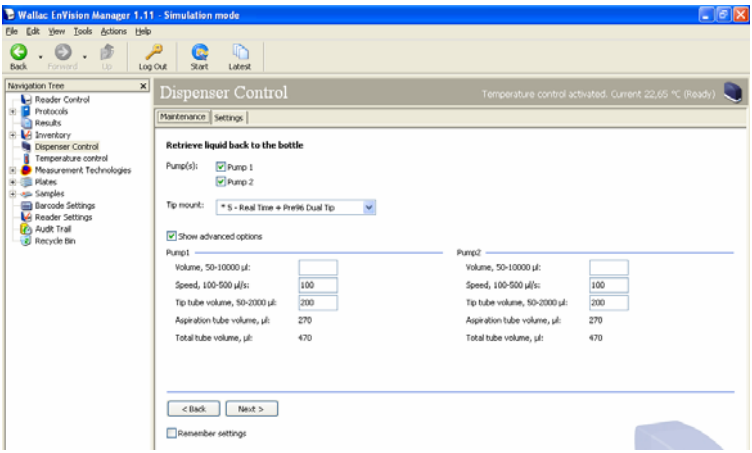
Click **Finish**. The **Maintenance** display will reappear.

Retrieve liquid



Returns liquid from the syringe and tubing to the liquid reservoir (use this if the liquid is expensive reagent!).

Click **Next** when you have selected this option.

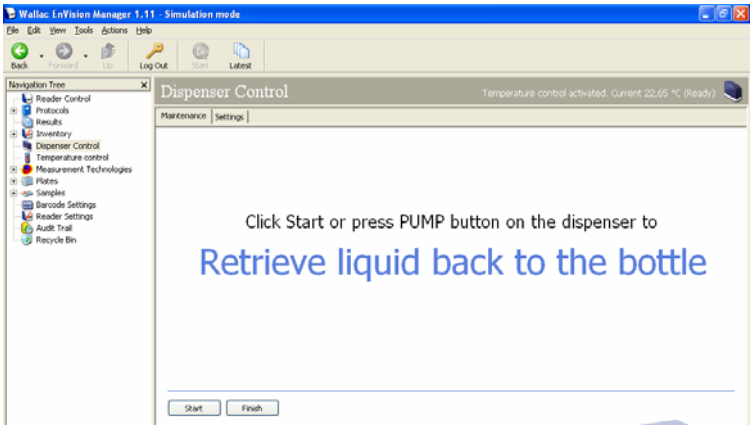


Select the pump or pumps from which you want to retrieve liquid.

Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

See **Show advanced options** for some extra parameters you can edit.

Click **Next**.



Click **Start** or press the PUMP button on the dispenser. Liquid will be retrieved to the liquid reservoir.

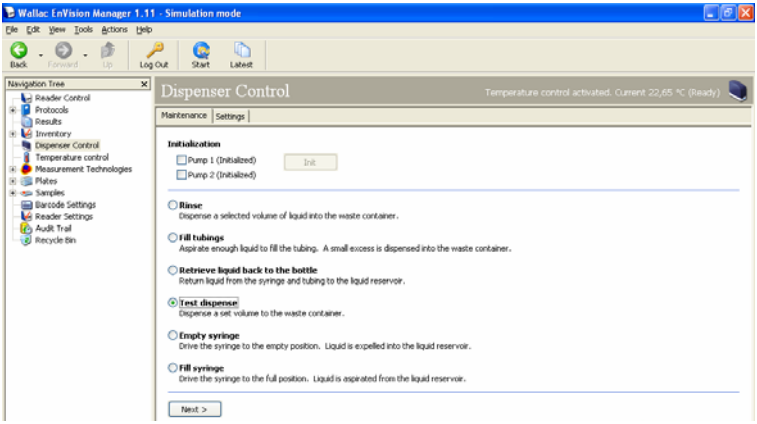
An animation will show what is happening.

You can repeat this operation if required to retrieve liquid from PUMP2.

Click **Finish**. The **Maintenance** display will reappear.

Test dispense

This operation allows you to dispense a set volume into the waste container.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Temperature control activated. Current 22,65 °C (Ready)
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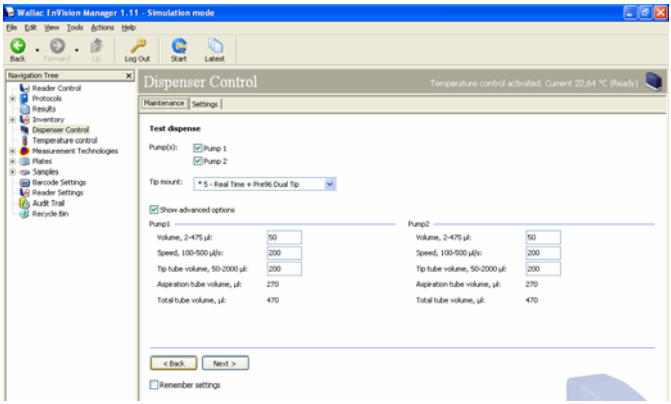
Pump 1 (Initialized)
Pump 2 (Initialized)
Init
Rinse
Dispense a selected volume of liquid into the waste container.
Fill tubings
Aspirate enough liquid to fill the tubing. A small excess is dispensed into the waste container.
Retrieve liquid back to the bottle
Return liquid from the syringe and tubing to the liquid reservoir.
Test dispense
Dispense a set volume to the waste container.
Empty syringe
Drive the syringe to the empty position. Liquid is expelled into the liquid reservoir.
Fill syringe
Drive the syringe to the full position. Liquid is aspirated from the liquid reservoir.
Next >

Click **Next** when you have selected this option.

Note! When the instrument lid is open, you can, by hand or with a tool, move the tip mount outside the instrument so that you can observe the flow of liquid through the tip. In this case the tip mount used must be identified.

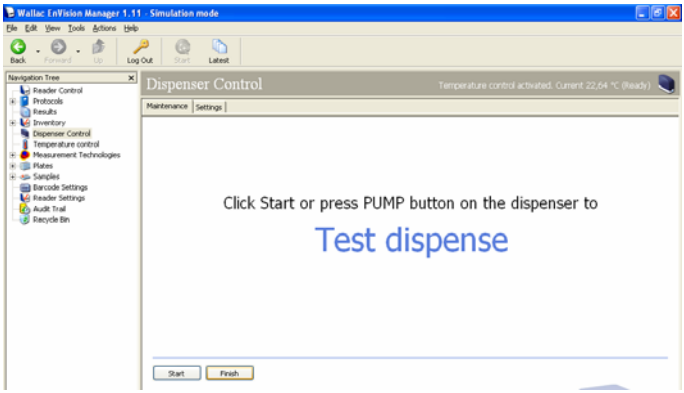


Select the pump or pumps with which you want to do a test dispense.

Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

See **Show advanced options** for some extra parameters you can edit.

Click **Next**.



Click **Start** or press the PUMP button on the dispenser. Liquid will be dispensed to the waste container.

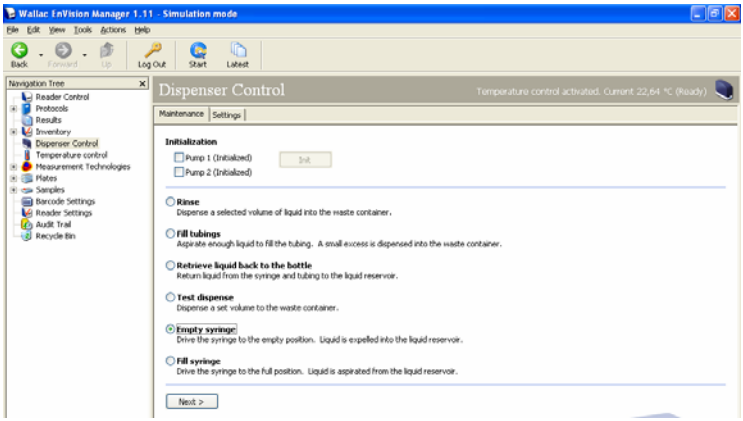
An animation will show what is happening.

You can repeat this operation if required to dispense liquid from PUMP2.

Click **Finish**. The **Maintenance** display will reappear.

Empty syringe

This operation drives the syringe to the empty position. Liquid will be expelled into the liquid reservoir.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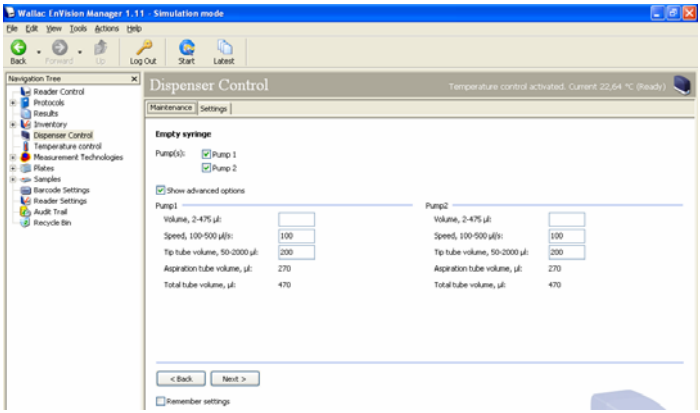
Pump 2 (Initialized)
Init
Rinse
Dispense a selected volume of liquid into the waste container.
Fill tubings
Aspirate enough liquid to fill the tubing. A small excess is dispensed into the waste container.
Retrieve liquid back to the bottle
Return liquid from the syringe and tubing to the liquid reservoir.
Test dispense
Dispense a set volume to the waste container.
Empty syringe
Drive the syringe to the empty position. Liquid is expelled into the liquid reservoir.
Fill syringe
Drive the syringe to the full position. Liquid is aspirated from the liquid reservoir.
Next >

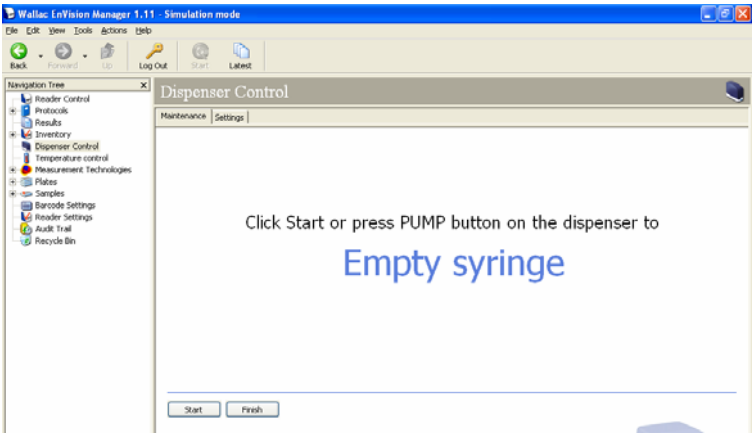
Click **Next** when you have selected this option.



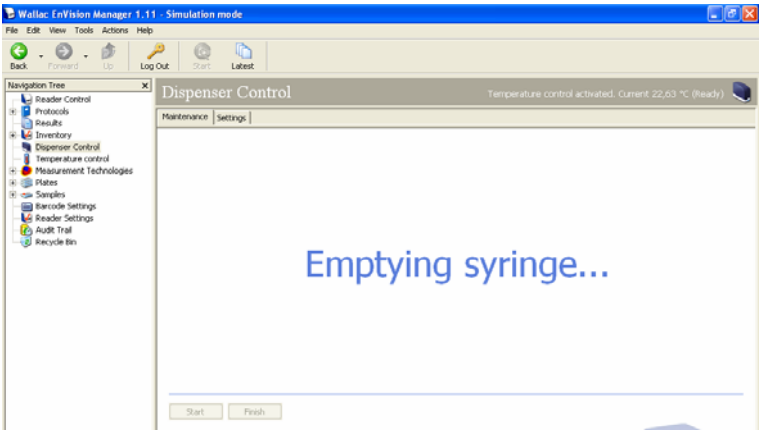
Select the pump or pumps with the syringe(s) you want to empty.

See **Show advanced options** for some extra parameters you can edit.

Click **Next**.



Click **Start** or press the PUMP button on the dispenser. The syringe will be emptied into the liquid reservoir.

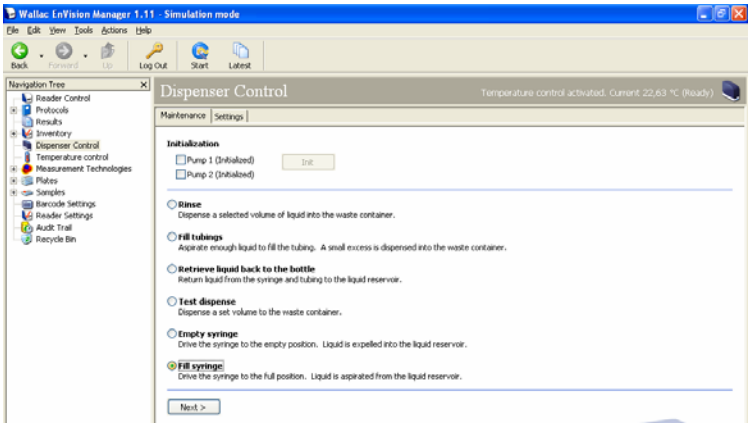


You can repeat this operation if required to empty the syringe of PUMP2.

Click **Finish**. The **Maintenance** display will reappear.

Fill syringe

This operation drives the syringe to the full position. Liquid will be aspirated from the liquid reservoir to fill the syringe.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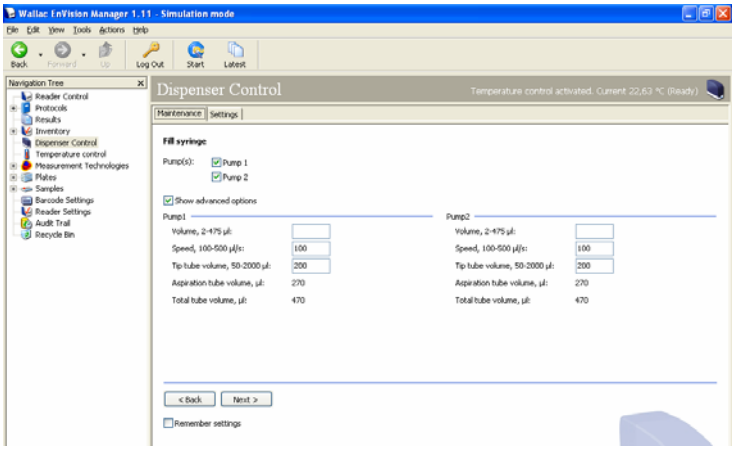
Temperature control activated. Current 22,63 °C (Ready)
Maintenance Settings
Initialization
Pump 1 (Initialized)
Pump 2 (Initialized)
Init
Rinse
Dispense a selected volume of liquid into the waste container.
Fill tubings
Aspirate enough liquid to fill the tubing. A small excess is dispensed into the waste container.
Retrieve liquid back to the bottle
Return liquid from the syringe and tubing to the liquid reservoir.
Test dispense
Dispense a set volume to the waste container.
Empty syringe
Drive the syringe to the empty position. Liquid is expelled into the liquid reservoir.
Fill syringe
Drive the syringe to the full position. Liquid is aspirated from the liquid reservoir.
Next >

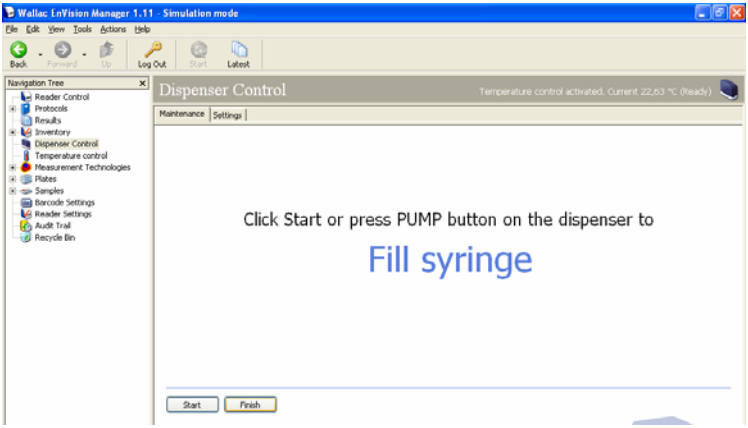
Click **Next** when you have selected this option.



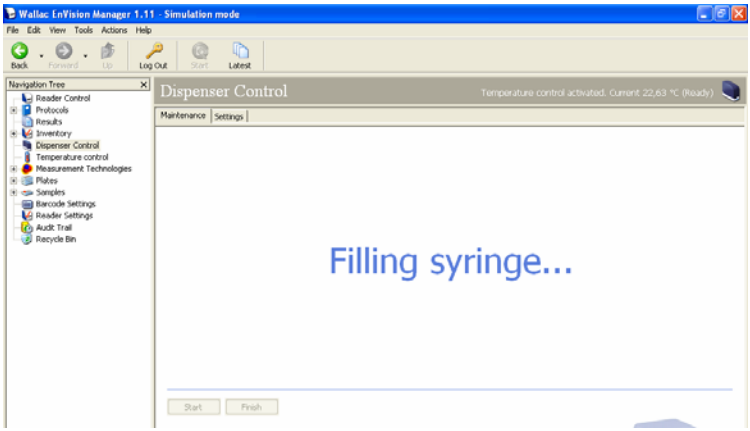
Select the pump or pumps with the syringe(s) you want to fill.

See **Show advanced options** for some extra parameters you can edit.

Click **Next**.



Click **Start** or press the PUMP button on the dispenser. The syringe will be filled from the liquid reservoir.

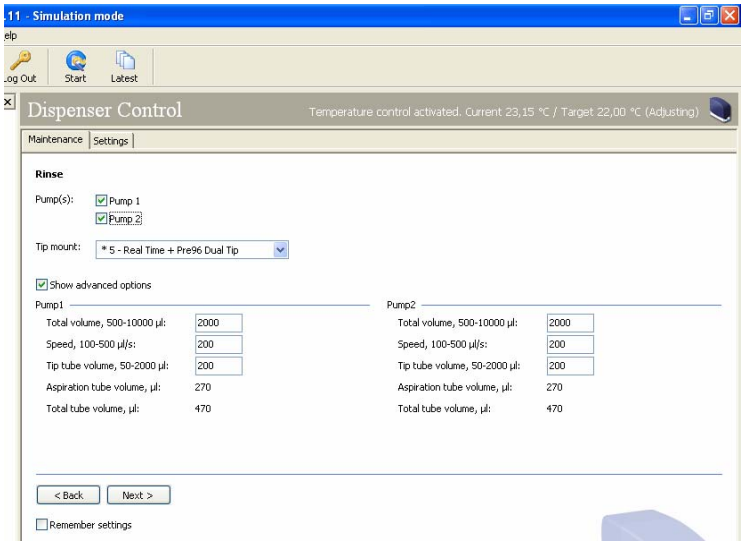


You can repeat this operation if required to fill the syringe of PUMP2.

Click **Finish**. The **Maintenance** display will reappear.

Show advanced options

Select **Show advanced options** if you want to see or change certain basic parameters. If you do not select this option then the default values will be used. There are two sets of identical parameters for Pump1 and Pump2.



(Total) Volume
This parameter determines the volume of liquid aspirated.

For **Rinse** the default is 2000 µl and the range 500 – 10000 µl. The parameter name is **Total volume** because several dispensings occur and this parameter is the total volume used for all the dispensings.

For **Fill** the default is 50 µl and the range 50 – 10000 µl. The parameter name is **Volume**.

For **Test** the default is 50 µl and the range 2 – 475 µl. The parameter name is **Volume**.

Speed 100 – 500 µl/s
This parameter determines the volume per second of liquid aspirated. The default is 200 µl/s. The more viscous the liquid the higher the speed you should use.

Tip tube volume 50 – 2000 µl
The volume of the tube between the syringe and the tip mount is known for a particular tip type and this volume appears as the default parameter value. The software allows you to change this value if it is necessary.

Aspiration tube volume µl
The value of this parameter is fixed here but can be changed under the **Settings** tab.

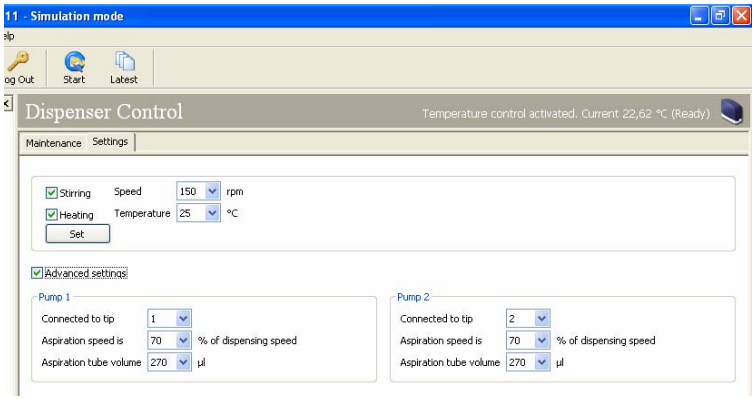
Total tube volume µl
This is the sum of the **Tip tube volume** and the **Aspiration tube volume**.

If you change these parameters and want to save the changes, select **Remember settings**.

Settings

Select the **Setting** tab to see these parameters.

Note! the following settings only take effect if you click the **Set** button.



Stirring
Select this check box and then select the speed to be used for stirring the liquid in the liquid reservoir. The range is 100 to 500 revolutions per minute.

Heating
Select this check box and then select the temperature for the liquid in the liquid reservoir. The range is 25 to 60 ºC.

Advanced settings
Select this to see extra parameters for both Pump1 and Pump2.

Connected to tip – select which tip you want each pump to be connected to: 1, 2 or None.

Aspiration speed is x% of dispensing speed – the range is 5 to 100% and the default 70%.

Aspiration tube volume – set the volume for the aspiration tube if it is different from the default 270 µl. The allowed range is 50 to 700 µl. This value is used in **Show**

Advanced options for the various dispense operations to calculate the **Total tube volume** by adding it to the **Tip tube volume**.

Dispenser guidelines

When starting operation

Perform **Initialization** when:
- you have switched on EnVision
- software is rebooted after being completely shut down
- you have pressed a PUMP button during dispenser operation

The software will tell you that you cannot run assays requiring dispensing if you have not initialized the system.

Before daily work

At the beginning of a working day we recommend that you perform the following operations depending on the situation:

- if the tubing has been rinsed and emptied when operation finished the previous time, (recommended procedure) then **Fill** the tubing with reagent.

- if reagent has been left in the tubing (not recommended), some may have been lost through evaporation. First **Retrieve** the reagent and then **Fill** the tubing.

- if rinse liquid has been left in the tubing, then you have to get rid of this before dispensing reagent. You can **Retrieve** the rinse liquid and then **Fill** the tubing. Alternatively you can empty the tubing using **Rinse** without aspirating any liquid i.e. with the inlet tube in air. Then replace the inlet tube in the reagent reservoir and **Fill** the tubing.

After operation

Empty syringe then **Retrieve** reagent back into the reagent reservoir. Replace this reservoir with one containing rinse liquid. **Rinse** the tubing. Either leave the tubing filled with rinse liquid or empty it i.e. **Rinse** with the inlet tube in air.

All these operations, as well as others, can be found under the **Maintenance** tab when you click ***Dispenser Control***.

Changing a tip mount

Note! before changing a tip mount, the tubing should be emptied. You can do this by selecting **Retrieve liquid** or **Rinse** with the inlet tube in air. To change a tip mount follow the instructions in the Routine maintenance part of the Instrument manual.

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